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# PATENT COOPERATION TREATY

## PCT

### NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

BROWN, Andrew, Stephen
Global Intellectual Property
AstraZeneca UK Limited
Mereside, Alderey Park
Macclesfield
Cheshire SK10 4TG
ROYAUME-UNI

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>19 September 2001 (19.09.01) | |
| **Applicant's or agent's file reference**<br>PHM70405/WO | **IMPORTANT NOTIFICATION** |
| **International application No.**<br>PCT/GB99/03330 | **International filing date** (day/month/year)<br>07 October 1999 (07.10.99) |

1. The following indications appeared on record concerning:

[X] the applicant   [ ] the inventor   [ ] the agent   [ ] the common representative

| Name and Address | State of Nationality | State of Residence |
|---|---|---|
| ASTRAZENECA UK LIMITED<br>15 Stanhope Gate<br>London W1Y 6LN<br>United Kingdom | GB | GB |
| | Telephone No. | |
| | Facsimile No. | |
| | Teleprinter No. | |

RECEIVED
OCT 30 2001
TC 1700

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person   [ ] the name   [ ] the address   [ ] the nationality   [ ] the residence

| Name and Address | State of Nationality | State of Residence |
|---|---|---|
| ASTRAZENECA AB<br>S-151 85 Södertälje<br>Sweden | SE | SE |
| | Telephone No. | |
| | Facsimile No. | |
| | Teleprinter No. | |

3. Further observations, if necessary:
**This Form cancels and replaces Form PCT/IB/306 issued on 28 July 2000.**

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>Dominique DELMAS |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

Form PCT/IB/306 (March 1994)

004297277

# PATENT COOPERATION TREATY

## PCT

**NOTIFICATION OF THE RECORDING OF A CHANGE**

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

BROWN, Andrew, Stephen
Global Intellectual Property
AstraZeneca UK Limited
Mereside, Alderey Park
Macclesfield
Cheshire SK10 4TG
ROYAUME-UNI

| | |
|---|---|
| **Date of mailing** (day/month/year) 22 August 2000 (22.08.00) | |
| **Applicant's or agent's file reference** PHM70405/WO | **IMPORTANT NOTIFICATION** |
| **International application No.** PCT/GB99/03330 | **International filing date** (day/month/year) 07 October 1999 (07.10.99) |

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

| Name and Address | State of Nationality | State of Residence |
|---|---|---|
| ASTRAZENECA UK LIMITED<br>15 Stanhope Gate<br>London W1Y 6LN<br>United Kingdom | GB | GB |
| | Telephone No. | |
| | Facsimile No. | |
| | Teleprinter No. | |

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

| Name and Address | State of Nationality | State of Residence |
|---|---|---|
| ASTRAZENECA AB<br>Global Intellectual Property<br>PO Box 272, Mereside<br>Alderley Park, Macclesfield<br>Cheshire SK10 4GR<br>United Kingdom | GB | GB |
| | Telephone No. | |
| | Facsimile No. | |
| | Teleprinter No. | |

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland**<br>Facsimile No.: (41-22) 740.14.35 | Authorized officer<br>Jean-Marie McAdams<br>Telephone No.: (41-22) 338.83.38 |

# PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

**PCT**

**NOTIFICATION OF ELECTION**

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>28 June 2000 (28.06.00) | |
| **International application No.**<br>PCT/GB99/03330 | **Applicant's or agent's file reference**<br>PHM70405/WO |
| **International filing date** (day/month/year)<br>07 October 1999 (07.10.99) | **Priority date** (day/month/year)<br>08 October 1998 (08.10.98) |
| **Applicant**<br>GARMAN, Andrew, John et al | |

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   13 April 2000 (13.04.00)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland**<br>Facsimile No.: (41-22) 740.14.35 | Authorized officer<br>Eugénia Santos<br>Telephone No.: (41-22) 338.83.38 |

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03330

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C12M3/00 C12N15/89

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12M C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 5 457 041 A (GINAVEN ROBERT O ET AL) 10 October 1995 (1995-10-10) the whole document --- | |
| A | WO 96 10630 A (UNIV RUTGERS) 11 April 1996 (1996-04-11) the whole document --- | |
| A | WO 98 28406 A (BROWN DAVID ;DAVIS BRIAN (US); GENESYSTEMS INC (US)) 2 July 1998 (1998-07-02) the whole document --- | |
| A | WO 91 05519 A (US ARMY) 2 May 1991 (1991-05-02) the whole document ----- | |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search
3 February 2000

Date of mailing of the international search report
10/02/2000

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Hornig, H



Form PCT/ISA/210 (second sheet) (July 1992)

.09/763825
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# PATENT COOPERATION TREATY

REC'D 23 FEB 2001
WIPO PCT

14

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>PHM. 70405/WO | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/GB99/03330 | International filing date *(day/month/year)*<br>07/10/1999 | Priority date *(day/month/year)*<br>08/10/1998 |
| International Patent Classification (IPC) or national classification and IPC<br>C12M3/00 | | |
| Applicant<br>ASTRAZENECA AB et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☒ Lack of unity of invention
   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   VI ☐ Certain documents cited
   VII ☒ Certain defects in the international application
   VIII ☒ Certain observations on the international application

| Date of submission of the demand<br>13/04/2000 | Date of completion of this report<br>21.02.2001 |
|---|---|
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Diez Schlereth, D<br>Telephone No. +49 89 2399 7488 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

**I. Basis of the report**

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
**Description, pages:**

1-26 as originally filed

**Claims, No.:**

1-17 as originally filed

**Drawings, sheets:**

1/22-22/22 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   **see separate sheet**

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-15,17 |
| | No: | Claims | 16 |
| Inventive step (IS) | Yes: | Claims | 1-5,17 |
| | No: | Claims | 16 |

| | | | |
|---|---|---|---|
| Industrial applicability (IA) | Yes: | Claims | 1-17 |
| | No: | Claims | |

2. Citations and explanations
   **see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:
**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
**see separate sheet**

**item IV.**

The separate inventions are:

(claims 1-15 and 17): a microfabricated cell injector and methods and devices which involve the use of said microfabricated cell injector.
(claim 16): use of a needle in the piercing and injecting of material into, or extracting material from, cells.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reason: it would appear that the novel and inventive concept behind claims 1-15 and 17 is that the microfabricated cell injector is provided with means (a **microfluidic channel**, see item VIII. below) for bringing cells into a position that enables said cells to be pierced by an injection needle, said needle being positioned at a fixed location. This feature, however, is not present in claim 16, which gives rise to an objection of lack of unity "a priori" (Rule 13.2 PCT).

However, in order to accelerate the procedure and since a complete Search Report has been issued by the International Search Authority, a written opinion is given for all groups of inventions.

**item V.**

1.) Reference is made to the following documents:

D1: US-A-5,457,041
D2: WO-A-96/10630
D3: WO-A-98/28406
D4: WO-A-91/05519

***claims 1-15 and 17***

2.) D1, which is considered to be the closest state of the art, discloses an array of microneedles extending from a support substrate (e.g. a silicon wafer). The shape and dimensions of the needles is chosen such that upon pressing the array against an outer

surface of the target tissue, the tips of the needles pierce the surface of said tissue (col. 1, l. 9-18; col. 3, l. 21-68; col. 4, l. 1-2, 39-67; col. 5, l. 1-6; Fig. 1).

The subject-matter of claim 1 differs therefrom in that **cells suspended in a fluid are impelled towards the injection wall and pierced by the injection needle**.

In the light of the closest prior art, the technical problem to be solved by the present application was to provide an alternative microfabricated cell injector.

The solution proposed in claim 1 (and 2-5, 7-12 as dependent thereon) can be considered novel and inventive within the sense of Art. 33 (2) and (3) PCT for the following reasons (see, however, item VIII. below):

The cell injector of claim 1 enables a large number of cells to be microinjected in a reliable way and with minimal operator involvement.

D2 discloses a method and a device for introducing biological material into cells. The method comprises providing (i) an array of truncated pyramidal microprobes positioned on a support (e.g. a silicon wafer), (ii) a solid or quasi-solid mass of target cells defining an interface with the microprobes, and (iii) the biological material placed at the interface, said material being introduced in the target cells by pressing the array of microprobes against the target cells (p. 3, l. 25-38; p. 4, l. 1-30; p. 7, l. 20-38; p. 8, l. 1-12; Figs. 1-2).

D3 discloses a method and device for microinjection of foreign material into non-adherent cells. The method comprises (i) providing an array of microneedles, (ii) supporting the cells onto a solid support, and (iii) microinjecting a solution of the foreign material into the supported cells (p. 1, l. 5-13; p. 7, l. 1-11; p. 8, l. 6-11; claims 25-27).

D4 discloses an injection device for introducing small amounts of material into a plurality of cells, which consists of a lower plate (e.g. a silicon chip) having an array of wells attached to an upper plate having a complementary array of injection needles. (p. 1, l. 3-6; p. 2, l. 24-32; p. 3, l. 1-3, 17-32; p. 4, l. 1-32; p. 8, l. 21-32; p. 9, l. 1-14; Figs. 1 & 4).

In the microfabricated devices of D1, D2 and D3 the needles are brought towards the cells, therefore, an uncertain (and unforseeable) number of target cells are pierced depending on the shape and configuration of the microneedle (or microprobe) array. In the device of D4, the wells of the lower plate can be dimensioned to hold a single cell which is retained in place by applying vacuum to the wells.

Therefore, the skilled person confronted with the above mentioned technical problem and equipped with the teaching of D1-D4 would have had neither motivation nor technical guidance for modifying the device of D1 in order to arrive at a cell injector according to claim 1, in which cells suspended in a fluid are impelled towards the injection wall (from which the injection needle projects) to bring the cells to a position which enables them to be pierced by the injection needle.

3.) The subject-matter of claim 6 (and 7-12 as dependent thereon) is considered novel and inventive within the sense of Art. 33 (2) and (3) PCT for analogous reasons as discussed in item 2. above (see, however, item VIII. below), the reasons being as follows. The cell injector of claim 6 comprises an internal surface defining a conduit which comprises an inlet, an outlet and an injection needle. These features lead to the same technical effect achieved with the cell injector of claim 1; namely, the cells are brought to a position which enables them to be pierced by the injection needle.

4.) The subject-matter of independent claims 13-15 and 17 relates to methods and devices which involve the use of the microfabricated cell injector of caims 1-12 and is considered novel and inventive within the sense of Art. 33 (2) and (3) PCT for analogous reasons as discussed in item 2. above (see, however, item VIII. below)

***claim 16***

5.) The subject-matter of claim 16 is not novel within the sense of Art. 33 (2) PCT, since it is anticipated by D4. This document, discloses the use of microneedles for injecting small amounts of material into cells. The needles are hollow, circular in cross section and the external diameter of the needle continuously decreases as it projects from the base of the needle (see Fig. 4). D4 discloses that the needles can be used to inject cells having a diameter of ca. 10 um (see p. 9, l. 9-14), therefore, this document implicitly discloses the use of needles with a diameter of less than 25 um.

**item VII.**

Contrary to the requirements of Rule 5.1 (a) (ii) PCT, the relevant background art disclosed in the documents D1-D4 is not mentioned in the description, nor are these documents identified therein.

**item VIII.**

The present set of claims does not meet the requirements of Art. 6 PCT for the following reasons:

1.) Independent claims 1, 6, 13 and 17 attempt to define a device (or a cell injector) by a manufacture process **"microfabricated cell injector/device"** instead of by technical features inherent to the cell injector/device. It would appear that a "microfabrication process" leads concomitantly to certain dimensions of the injection needle, as stated on page 4, lines 1-4. Since the "microscale" dimensions of the injection needle is an essential feature for defining the invention, and claims 1, 6, 13 and 17 do not clearly refer to this feature, they do not meet the requirement following from Art. 6 PCT taken in combination with Rule 6.3 (b) PCT (PCT Guidelines III-4.3).

2.) The subject-matter of independent claim 1 is not clearly defined as required by Art. 6 PCT. The applicant attributes a special meaning to the term **"injection wall"** (<u>the injection needle is positioned on a surface of the microfluidic channel, the injection wall, which forms part of the housing for the needle</u>, see p. 4, l. 10-14), which cannot be inferred from the wording of the claim (PCT Guidelines III-4.2). Since this feature is essential for carrying out the invention, the claim in its present form does not meet the requirement of Art. 6 PCT in combination with Rule 6.3 (b) PCT in that the claim must contain all technical features essential to the definition of the invention.

Furthermore, the claim attempts to define the subject-matter in terms of the result to be achieved (cell injector comprising ... such that cells are impelled towards...) which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result (the cell injector is provided with cell propulsion means, see claim 2) should have been included in the wording of the claim (PCT Guidelines III-4.7).

3.) The expression **"propulsion means"** does not have a precise meaning for the skilled person and renders the subject-matter of claim 2 unclear (Art. 6 PCT). A clear r definition of this concept should have been included in the wording of the claim (PCT Guidelines III-4.2).

4.) The subject-matter of claims 3-5 is not clearly defined as required by Art. 6 PCT. The applicant attributes a special meaning to the term **"housing"** (the housing encloses a cell injection needle and a microfluidic channel, see page 4, lines 7-14) which cannot be inferred from the wording of the claim. A clearer definition of this term should have been included in the claim (PCT Guidelines III-4.2).

5.) In independent claim 6 , the applicant attributes a special meaning to the wording **"internal surface defining a conduit"** (a microfluidic channel?), which cannot be inferred from the wording of the claim (PCT Guidelines III-4.2). Since this feature is essential for carrying out the invention, the claim in its present form does not meet the requirement of Art. 6 PCT in combination with Rule 6.3 (b) PCT in that the claim must contain all technical features essential to the definition of the invention.

Furthermore, the claim attempt to define the subject-matter in terms of the result to be achieved (cell injector comprising ... such that cells enter ... are moved along ... and are pierced) which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result (cells are pierced by the cell injection needle when the presence of a cell nearby the needle is detected by a cell sensor, see claim 7) should have been included in the wording of the claim (PCT Guidelines III-4.7).

6.) The wording "device containing a plurality" used in claim 13 renders the scope of said claim unclear, since the only feature of the device which is disclosed in this application is the (or the plurality of) cell injector(s). Therefore, the claim is not supported by the description in its full breadth (Art. 6 PCT, PCT Guidelines III-6.1).

7.) The wordings of claims 14-16 do not unambiguously exclude the possibility to carry out the methods (or tu use the needle in a method) "in vivo". A clarification of the "in vitro" nature of the methods should have been included in the wording of the claims in order to comply with Rule 67.1 (iv) PCT (PCT Guidelines IV-2.4 (d)).

8.) In order to meet the requirements of Rule 13 PCT regarding unity of invention, the method of claim 14 should have been specially adapted to be carried out with a microfabricated cell injector device according to claims 6-12 (PCT Guidelines III-7.3). This feature, however, is not specified in claim 14. This results in a lack of clarity as regards essential technical features required for carrying out the invention (Art. 6 PCT, PCT Guidelines III-4.3) and also a lack of clarity as regards the common inventive concept linking claims 1, 6, 14 and 17 (Rule 13.1 PCT).

9.) The expression "substantially" renders the subject-matter of claim 16 unclear (Art. 6 PCT, PCT Guidelines III-4.5a). Furthermore, technical features following the expression "preferably" have no limiting effect on the scope of claim 16 and are to be regarded as entirely optional (Art. 6 PCT, PCT Guidelines III-4.6).

10.) The wording "combined with one or more process or analysis steps" used in claim 17 is vague and renders the subject-matter of said claim unclear (Art. 6 PCT). Consequently, the claim is not supported by the description in its full breadth (Art. 6 PCT, PCT Guidelines C III 6.1).

M·H

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference<br>PHM70405/WO | **FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
|---|---|---|
| International application No.<br>PCT/GB 99/ 03330 | International filing date *(day/month/year)*<br>07/10/1999 | (Earliest) Priority Date *(day/month/year)*<br>08/10/1998 |
| Applicant<br>ZENECA LIMITED et al. | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

      [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

      [ ] contained in the international application in written form.
      [ ] filed together with the international application in computer readable form.
      [ ] furnished subsequently to this Authority in written form.
      [ ] furnished subsequently to this Authority in computer readble form.
      [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
      [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.
   [X] the text has been established by this Authority to read as follows:

   MICROFABRICATED CELL INJECTOR

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 14

   [ ] as suggested by the applicant.
   [X] because the applicant failed to suggest a figure.
   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

Form PCT/ISA/210 (first sheet) (July 1998)

# INTERNATIONAL SEARCH REPORT

International Application No
T/GB 99/03330

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C12M3/00 C12N15/89

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12M C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 5 457 041 A (GINAVEN ROBERT O ET AL) 10 October 1995 (1995-10-10) the whole document --- | |
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/03330

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| WO 9828406 A | 02-07-1998 | AU 5618298 A | 17-07-1998 |
| | | EP 0948594 A | 13-10-1999 |
| WO 9105519 A | 02-05-1991 | US 5262128 A | 16-11-1993 |
| | | AU 6640190 A | 16-05-1991 |
| | | EP 0497885 A | 12-08-1992 |

**CORRECTED VERSION***

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

**(51) International Patent Classification 7 :** C12M 3/00, C12N 15/89 — A1

**(11) International Publication Number:** WO 00/20554

**(43) International Publication Date:** 13 April 2000 (13.04.00)

**(21) International Application Number:** PCT/GB99/03330

**(22) International Filing Date:** 7 October 1999 (07.10.99)

**(30) Priority Data:**

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| 9918614.0 | 7 August 1999 (07.08.99) | GB |

**(71) Applicant** *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).

**(72) Inventors; and**
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**(74) Agent:** BROWN, Andrew, Stephen; Global Intellectual Property, Patents, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).

**(81) Designated States:** AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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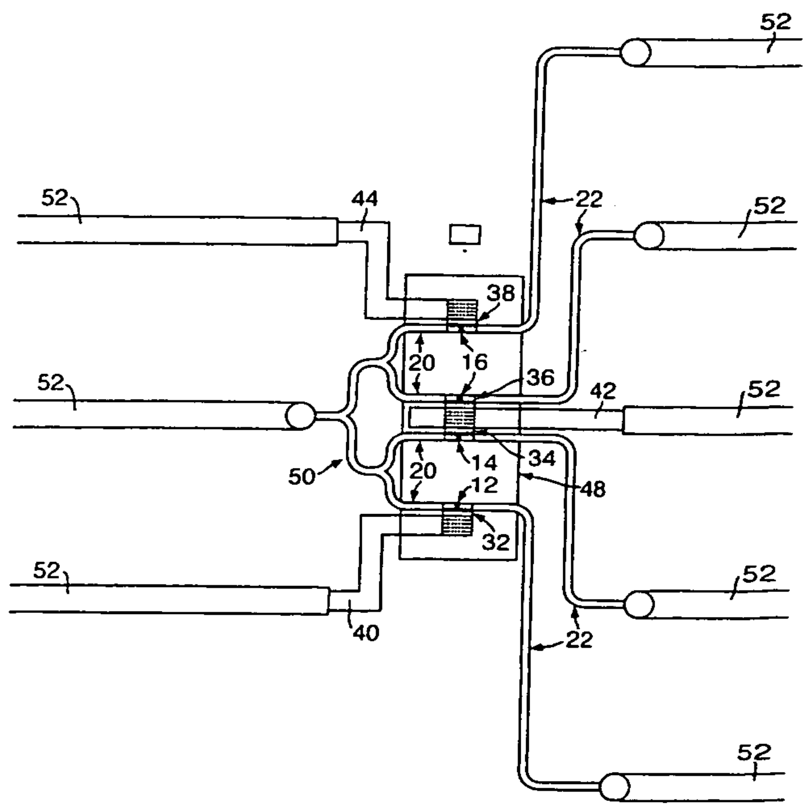
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

**(54) Title:** MICROFABRICATED CELL INJECTOR

**(57) Abstract**

The invention relates to a device for, and method of, injecting small articles, in particular cells. More particularly, but not exclusively, the invention relates to an automated device for and method of injection of large numbers of cells. The invention also includes use of such a device, specifically in fields where low throughput of cell injection from current techniques has meant that such uses have not been viable.

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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03330

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C12M3/00 C12N15/89

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12M C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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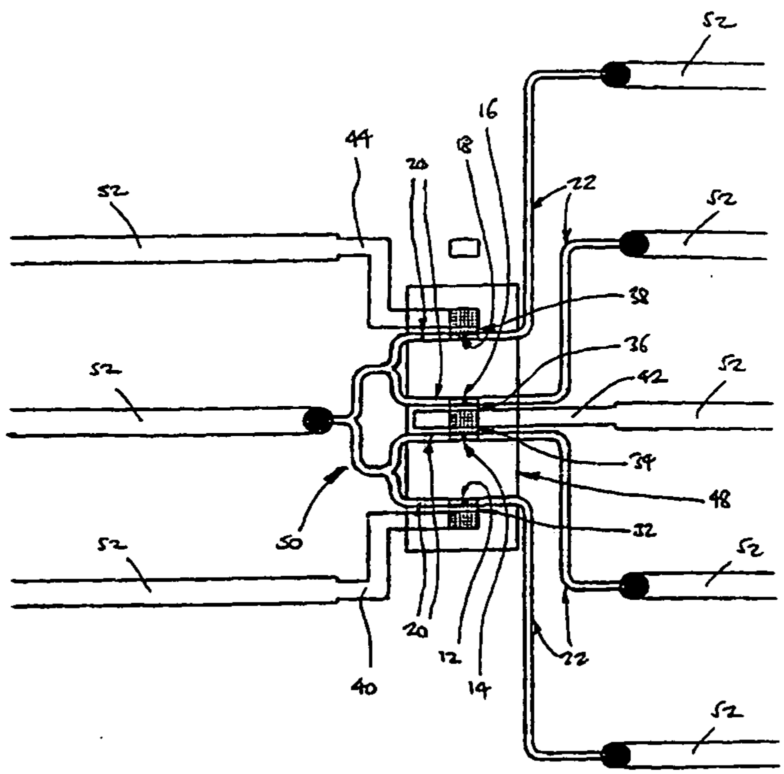

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MICROFABRICATED CELL INJECTOR

The invention relates to a device for, and method of, injecting small articles, in particular cells. More particularly, but not exclusively, the invention relates to an automated device for and method of injection of large numbers of cells. The invention also includes use of such a device, specifically in fields where low throughput of cell injection from current techniques has meant that such uses have not been viable.

Injection of cells is currently only a viable technique in a limited number of fields, for example *in vitro* fertilisation, and currently is carried out manually and individually on each cell. It requires a high level of skill and an experienced operator can only inject in the order of one cell per minute. There are many other fields that would benefit from cell injection of macromolecules, genes, chromosomes, organelles, or any other substance desired to be injected into a cell were it possible to achieve this on a large numbers of cells. Gene therapy, biotechnology, life sciences research, diagnostics, pharmaceutical and agrochemical research are among many fields that would benefit from a more facile high throughput cell injection method.

Currently using manual techniques the cells are suspended in solution and each cell is individually injected by fixing a cell into position by the operator "sucking" the cell onto the end of a narrow pipette. Whilst watching the operation through a microscope the operator then inserts a needle into the cell. Once the injection is made the needle is retracted manually and the cell released, then the next cell is injected and so on. In addition variations of this basic manual technique are available such as, for example, for injecting cells which are attached to a dish as a monolayer. The cost of injecting a small number of cells is expensive and means that microinjection of cells is not a technique used widely in the pharmaceutical or agrochemical research.

We have devised of a device in which a large numbers of cells (hundreds, thousands or millions) may be micro-injected with minimal operator involvement by use of a microfabricated device which impels cells onto an injection needle.

Microfabrication techniques are generally known in the art using tools developed by the semiconductor industry to miniaturise electronics and it is possible to fabricate intricate fluid systems with channel sizes as small as a micron. These devices can be mass-produced inexpensively and are expected to soon be in widespread use, for example, in simple analytical tests. See, e.g., Ramsey, J.M. et al. (1995), "Microfabricated chemical measurement Systems," Nature Medicine 1:1093-1096; and Harrison, D.J. et al (1993), "Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip," Science 261:895-897.

Devices made by micromachining planar substrates have been made and used for chemical separation, analysis, and sensing. See, e.g., Manz, A. et al. (1994), "Electroosmotic pumping and electrophoretic separations for miniaturized chemical analysis system," J. Micromech. Microeng. 4:257-265.

We have been able to construct a microfabricated needle onto which cells may be impelled towards so that the needle pierces the cell and material may be injected or extracted from the cell.

We present as a first feature of the invention a microfabricated cell injector comprising an injection wall and projecting from the injection wall a cell injection needle, such that in use cells suspended in a fluid are impelled towards the injection wall and pierced by the injection needle whereupon material is (1) injected into the cell, (2) extracted from the cell, or (3) injected into the cell and then extracted from the cell the steps being in any order and any number of times.

We have also found that by careful arrangement of channels (microfluidic channels) formed within a microfabricated device a conduit is formed through which the flow of cells in a suspension may be controlled to an extent that cells may be injected by impelling them onto an injection needle individually.

We disclose as a further feature of the invention a microfabricated cell injector comprising an internal surface defining a conduit,which in use transports cells suspended in a

fluid, the conduit having an inlet and an outlet, the conduit further comprising a cell injection needle, such that, in use cells enter the injector via the inlet, are moved along the conduit and are pierced by the cell injection needle whereupon material is (1) injected into the cell, (2) extracted from the cell, or (3) injected into the cell and then extracted from the cell the steps being in any order and any number of times, and the cells are then, optionally, moved to the outlet.

A further feature of the invention is a method for the microinjection of cells which method comprises passing a suspension of cells in a fluid through a conduit comprising a cell injection needle, the cells thereby being pierced by the injection needle and material is: (1) injected into the cell (2) extracted from the cell or (3) injected into the cell and then extracted from the cell the steps being in any order and any number of times; as the cells pass through the conduit.

It should be understood that the arrangement, type and dimensions of the device and the components therein will vary according to the use or application, as will become apparent. It is generally preferred that the microfabricated conduit only allows a single cell to be impelled upon a single injection needle at any one time.

In this disclosure, the term "microfabricated" includes, for example, devices capable of being fabricated on glass, plastic, silicon or any other suitable material. Suitable microfabrication techniques are those readily available to those practising the art of microfabrication and include such methods as LIGA, thermoplastic micropattern transfer, resin based microcasting, micromolding in capillaries (MIMIC), wet isotropic and anisotropic etching, laser assisted chemical etching (LACE), and reactive ion etching (RIE), or other techniques known within the art of microfabrication. In the case of silicon microfabrication, larger wafers will accommodate a plurality of the devices of this invention in a plurality of configurations. A few standard wafer sizes are 3" (7.5cm), 4"(10cm), 6"(15cm), and 8"(20cm). Application of the principles presented herein using new and emerging microfabrication methods or materials is within the scope and intent of the invention.

The injection needle has a diameter of dimensions comparable with the dimension of the cells to be injected, for example between 1% and 50% of the cell diameter, preferably between 5% and 30%. Typical cell diameters are from 10 microns to 50 microns, but will vary according to the cell origin and type. The walls of the injection needle when hollow may be from 1 micron thick and may be as narrow as 0.1 micron thick at the point of the needle. Where the injection needle has a hollow tip this is connected to a microfluidic channel which is able to deliver to the injection needle tip the material for injection. Preferably the injection needle is fixed in the device relative to the walls of the microfluidic channels which form the conduit such that it projects and injection is achieved by moving the cells on and off the injection needle, rather than by moving the injection needle into and out of the cell. Preferably the injection needle is positioned on a surface of the microfluidic channel, which we term the "injection wall" , see for example Figure 1. The injection wall forms part of the "housing" for the needle which may be simply an integral part of the conduit or may be a distinct aspect which forms a suitable receptacle for receiving/positioning/holding the cell prior to injection. The length and shape of the injection needle that is exposed above the injection wall will determine the "injection depth", that is the depth to which the injection needle will penetrate the cell. This depth will depend on the cell type, the design of the needle, and the application. In particular, it will depend on the cellular compartment that it is desired to inject into. For example, for injection into the cytoplasm, the injection depth could be in the order of 1 micron, for example 1 to 5 microns; whereas, for an injection into the nucleus, the injection depth will need to be greater, for example 3-10 microns. Given a knowledge of the cell type, it will be possible for the skilled bioscientist to select a device with the appropriate injection depth.

Preferably the needle is hollow and substantially circular in cross section, the external diameter of the needle continuously decreasing as it projects from the base of the needle to its tip, the tip being less than 25 microns, preferably less than 5 microns, in diameter. As a feature of the invention we present use of the above described needle in the piercing and injecting of material into, or extracting material from, cells.

The injection wall surrounding the immediate area of the injection needle may be permeable to the medium in which the cells are contained, but impermeable to the passing of cells. In certain orientations of the device permeable walls are preferred and allow the passing

of the cell medium through the injection wall, facilitating the movement of the cell towards the injection needle. The permeability of the wall may be achieved by one or a small number of orifices positioned around the needle, preferably in a symmetrical fashion. It will be clear that a large number of designs could in principle achieve the aim of forcing the cell onto the needle and these are incorporated into the invention. The injection wall may optionally itself be charged to attract the cell towards the injection needle, or reverse charged to expel the cell from the injection needle. Alternatively the charge may alternate to impel the cell onto the needle and then to expel it. The injection wall may be flat or any other shape to accommodate the cell whilst it is on the injection needle.

Embodiments of the invention are now described , by way of examples only and with reference to the Figures, in which:
The invention is described in more detail below in the following non-limiting figures.

Figure 1 is a diagrammatical cross-sectional view of a needle injecting a small article, such as a cell;
Figure 2 shows diagrammatically views of a cell approaching, impinging and being removed from a needle;
Figure 3 and 4 are diagrammatical representations of an injection system, enabling auotmatic delivery and removal of a cell;
Figure 5 is a diagrammatical view of an alternative arrangement for a cell injector;
Figure 6 is a diagrammatical view of a cell injector device having a number of cell injector units run in parallel.
Figure 7 is a diagrammatical view of a needle housing;
Figure 8 is a diagrammatical view of an injection device showing the needle and housing.
Figure 9 is a photograph taken through an electron microscope of a cell injector needle; and
Figure 10 to 16 show methods to produce the devices.
Referring to the figures generally, and specifically to Figure 1, the depth of entry of the injection needle **2** into the cell **1** is defined by the distance the cell may travel before it is stopped at the cell injection wall **5**. Material **3** may be injected into the cell by pulsed injection when the cell is in position, or by continuous flow where the time the cell is spent on the injection needle is regulated.

Figure 2 - Shows one alternative method for impelling the cell onto the injection needle and removal once injection has taken place. The cell is impelled onto the injection needle **1** and **2** by a passive force, i.e. the liquid and cell moves, or an active force, i.e. where the cell moves. Movement of the cell onto the injection needle is eased by providing a permeable cell injection wall for the cell suspending liquid to pass through. The cell is removed from the injection needle by providing an opposite active or passive force **3**.

Figure 7 - Shows an alternative arrangement for the injecting wall is at the base of the needle and the narrowing channel which supports the cell **1** during injection is the housing.

The injection needle may be a fabricated separately from the microfabricated conduit and inserted during manufacture into the device. Alternatively the injection needle is fabricated during the manufacture of the conduit and the injection needle formed as a simple projection from the surface of the injection wall, see for example Figure 8.

The design of the needle is conveniently conventional that is comprising a single hollow tube, preferably sharp at the point and optionally widening towards the base. However, other structures are included. We include structures where the penetration of the cell and the injection of fluid are achieved by different parts of the structure. For example, the point of the needle may be solid and injection of fluid carried out by means of side channels. Alternatively, the needle may be a simple point made out of a porous material, the injected material entering the cell by means of the pores. Alternatively, the needle is a solid point for piercing the cell, preferably along one side of the needle a groove, and the material present in the suspension fluid is allowed to flow into the cell.

As an alternative to a cell piercing needle as described above a non-cell piercing needle or area may be used in which the cell is held against and from which a membrane-opening chemical or force is applied.

The choice of needle design will depend on factors such as the size of the cell, the type of cell, the desired efficiency of injection (measured as the percentage of cells injected or the percentage of injected material that is injected).

The injection needle is conveniently an essentially rigid structure, that is it does not move significantly with respect to the rest of the device. However, depending on the microfabrication approach employed, it may be convenient for the needle to be flexible, i.e. movement is allowed in the direction of the needle axis. Optionally this flexibility may be exploited to augment the needle penetration pressure, either due to the elasticity of the structure, or differences in hydrostatic pressure, or both.

Figure 9 - Is a photograph taken through an electron microscope of a cell injector needle, magnification x1500, The needle is 2 microns at the top and the walls are 0.1 micron thick. The silicon slab is back-etched so that there is a hole right through the structure which is used for injection of material into the cell.

It is convenient for the channel that provides the driving force impelling the cells onto the needle to be defined in part by the plane in which the needle structure is fabricated.

The conduit may comprise any number of microfluidic channels within the device so as to form an opening for entrance of cells into the device and the conduit being in fluid communication with a second opening for cells to exit the device. The microfluidic channels may be of any suitable size and dimensions which allow cells suspended in a fluid to flow from the entrance to the exit via the cell injecting needle. Diameters of the microfluidic channels may vary along the conduit but will typically range from 10 microns to 300 microns, preferably 30 microns to 100 microns.

Optionally the cells are accelerated onto the needle by means of a restriction in the microfluidic channel which increases the linear velocity of the cell. Alternatively the walls of the channel could be fashioned to compress the cell to assist with the injection.

Cells may be suspended in any fluid which is able to flow readily through microfluidic channels and not adversely harm the cells suspended within the fluid. Preferred fluids are buffered aqueous solutions, at physiological pH, optionally containing cell nutrients to preserve cell viability.

The method / device of the invention includes active propulsion of the cell onto the needle, in addition to or instead of the forces provided by movement of the cell suspension. By active propulsion we include provision of a pressure behind the cell (opposite side to the needle) by means of a deformable section of the channel wall, such deformation being achieve by externally applied pressure such as gas pressure, liquid pressure or mechanical pressure. We also include provision of a negative pressure ahead of the cell (same side as the needle) by the same or similar means. We also include an additional, optionally externally applied, positive or negative pressure pulse in the carrier liquid in which the cells are suspended. We also include other external forces such as magnetic or electrostatic fields acting on an appropriately derivatised cell suspension. Cells may be moved along the microfluidic channels and impelled onto the injection needle by any convenient means. Two broad categories are envisaged. Firstly is passive flow, where the carrier fluid containing the cells of interest is moved and the cells flow with it. The carrier fluid may be propelled by means of a mechanical pump, by applying a vacuum or pressure to one end of the channel, by gravity flow, by electro-osmosis or any other suitable means. Electro-osmosis, which may conveniently be achieved by the microfabrication of electrodes at the ends of the channels, the voltages between electrodes being controlled conveniently externally to achieve the desired fluid movements. Alternatively active flow may be employed where the cell is moved actively, i.e. independently of the carrier fluid by means of an external field, for example an electrostatic field. Means of manipulating cells by various types of electric field are described in the literature, see for example R.Pethig and G.H.Markx, Trends in Biotechnology (1997) 15, 426-. Combinations of methods are also possible, for example, the cells may be delivered to the injection area of the device by passive flow and then impelled by an active force onto the injection needle.

The above cell movement methods are may also be used, optionally in combination, to remove the cell from the injection needle and to flow the cells away and out of the device.

Where the cells are manipulated onto or off the injection needle by passive flow, the injection wall may alternatively be constructed with holes or small channels therein, or is of a porous material, so that fluid can flow relatively unhindered through the wall. Figure 2 illustrates an example of an arrangement capable of achieving this.

By high throughput we mean that the invention can achieve a throughput substantially higher than conventional means and that numbers of cells in the orders of 100, for example 1,000 or 1 million, can be achieved in a convenient time period in the order of minutes or hours. In order to achieve the higher throughputs, the method optionally involves parallel processes, i.e. multiple devices are used in parallel and cells are flowed along a plurality of microfluidic channels such that they are impelled onto a plurality of injection needles, for example, one injection needle per channel. Figure 6 shows such a device with 8 channels in parallel. A requirement of such devices, which we term "multi-channel" is that the inlets of the multiple injector units are connected to suitable channels to divide up the flow of cells from a cell sample reservoir, then, preferably, recombined after injection, and the material for injection is also divided up by suitable channels to provide material to each injection area.

Accordingly we disclose as the second feature of the invention a microfabricated device containing a plurality of cell injector units, each cell injector unit comprising a conduit or an internal surface defining a conduit for transporting cells suspended in a fluid, and having an inlet and an outlet, the conduit further comprising a cell injection needle, such that in use cells enter the injector via the inlet and are moved along the conduit, pierced by the injection needle whereupon material is injected into the cell or extracted, and then moved to the outlet, the respective inlets and outlets of the cell injecting units being each connected such that the cells are divided into each injector unit and, preferably, recombined after injection.

The injection material is any material that it is desired to inject into the cell. Most advantageously, this is material that cannot readily be taken up by the cell of interest by any other convenient means. In particular, the material for injection is a macromolecule in aqueous solution, for example a peptide, protein, nucleic acid or polysaccaharide, and analogues and conjugates thereof. Also the injection material may comprise particles, for example viruses,

chromosomes, synthetic particles optionally containing or coated with a macromolecule of interest, spores, plasmids, cell organelles, vesicles, liposomes, micelles and emulsions. Optionally a label, for example a fluorescent label, may be added to the injection fluid to act as a marker to indicate that the injection is successful. Ideally the material is suspended or dissolved in a liquid, such as an aqueous buffer.

The arrangement of microfluidic channels to effect the method of the invention will depend on many factors such as the desired throughput and means of propelling cells.

Figure 3 - Is a diagram of a microfabricated cell injector with a conduit consisting of two channels crossing in opposing directions. Cells are impelled down channel A to D stepwise by an oscillating force which switches cell movement from A to D then D to A, the movement of cells from A to D being larger then the return movement and impelling a cell onto the injection needle 1. The smaller returning force D to A releases the cell from the injection needle after injection and places it in line with channel B to C. The cell is moved along B to C and then a force applied again to impel the next cell onto the injection needle in direction A to D.

In the case of passive flow and use of a porous injection wall, a convenient configuration is shown in Figure 3. Here a stream of cells enters along channel A (at this point in the cycle there is no flow between arms B and C) and the leading cell becomes impaled on the injection needle. A "capture sensor" senses that a cell is captured and the flow is halted and the material for injection is injected. The flow is immediately reversed in a short pulse which dislodges the cell. The strength and duration of this pulse is selected such that the cell is delivered to the centre of the cross. Flow from arm B to arm C (the reverse is possible) is initiated and the cell is removed along arm C. The cycle then starts again with flow along channel A which capture the next cell, the said cycles being repeated until the desired number of cell have been injected.

Alternatively, cells may be introduced along arm B and moved along arm A, as is illustrated in Figure 4.

Figure 4 - Shows an alternative arrangement of the microfabricated cell injector of figure 3 where the cells are moved along channel B to C in a stop/start motion where a cell placed in-line with channel A to D during the stop phase is impelled onto the injection needle by a force A to D and removed by a force D to A and placed back in line with the channel B to C and removed by the next movement in channel B to C.

Suitable capture sensors include a conductivity sensor that measures the conductivity or capacitance between the injection fluid and the carrier fluid. Conductivity changes when the needle penetrates the cell. Optionally, the change in impedance may be measured to detect a cell that is adhered to the needle but not actually penetrated by it. Alternatively, the capture sensor may take the form of a pressure transducer positioned near the point of capture such that capture causes a partial blockage of the flow in the pressure transducer and a change in pressure. Alternatively, the capture sensor may take the form of a pair of electrodes positioned either side of the cell when in the injection position, the electrodes being able to measure changes in conductivity or permittivity or another convenient electrical or magnetic parameter. Alternatively, optical methods may be used to image the cell or detect its presence at the injection position by changes in absorbance, refractive index, light scattering and the like. In the case of a flexible needle, pressure sensors may be used to detect the presence of a cell.

It will be appreciated that, depending on the cell density, density may vary from ......to ..... and the efficiency of injection required, it may be desirable to have a means of detecting the presence of a cell as it approaches or enters the injection area, i.e. a "cell sensor". This may be achieved by any convenient means for example by a pair of electrodes positioned either side of the cell when in the position for detection, the electrodes being able to measure changes in conductivity or another convenient electrical parameter. Alternatively, optical methods may be used to image the cell or detect its presence at the desired position by changes in absorbance, refractive index, light scattering and the like.

Optionally the device will be controlled by a "controller" which will monitor and coordinate the arrival and movement of cells in the injection unit. The controller will

automatically adjust the flow of cells in the various chambers to co-ordinated the arrival of cells at the injection needle.

Velocity of the cells as an active or passive velocity will be in the order of 10micron/sec to 10mm/sec, preferably 100micron/sec to 1mm/sec

A further way of impelling the cells onto the injection needle is by deflecting the flow at a substantial angle, for example between 40 degrees and 90 degrees such that the cell impacts a defined area of the channel wall which contains the injection needle. Removal of the cell is achieved by a combination of the elasticity of the cell bouncing off the wall and the flow in the microfluidic channel, optionally enhanced by a tumbling action of the cell initiated by geometric features fabricated in the channel wall. A non-limiting illustration of a device including this arrangement is shown in Figure 5.

Figure 5 - Is a diagram of a microfabricated cell injector with a conduit consisting of a single channel **1**. Cells impelled through the channel are forced onto an injection needle **4** by a deflector **2**. The momentum of the cell is sufficient for it to be impelled onto and then off the injection needle.

The walls of the channel are designed such that each cell is presented to the injection needle with the correct force such that the injection needle penetrates the cell wall, and that the cell then bounces off the wall and continues in the same direction down the channel, without tearing or otherwise irreparably damaging the cell wall. To achieve this, the channel walls have several features to achieve this. We here define the various surfaces and features (see Figure 5).

a "deflector wall", which causes the liquid flow and the cells to deviate
a "constrictor" which squeezes the cell slightly and increases the speed of the cell. The constrictor also encourages the cell to proceed down the channel with a tumbling action.

When the cell is positioned on the needle then injection of the material takes place. The material injected will depend on the cell type, volume and purpose of the injection. It will

normally be in the range of 1% to 50% of the cell volume, for example 5% to 20%, and will typically be in the order of one or a few picolitres. Movement of the material in the injection channel may be achieved by any convenient means and may include for example a micropump or piezoelectric displacement, such as described in Transaction on Biomedical Engineering (1975) 22, 5, 424-426. The movement may optionally be continuous where it is acceptable for material to leak into the carrier fluid and where this is not economically prohibitive.

Devices may be made by the use of microfabricated layers such as shown in Figure 8. Here the cells travel in suspension down a channel defined by the gap between layers 3 and 4, and by walls perpendicular to the layers, formed from layer 3 or 4 or both. As each cell enters the injection area, flow away from the injection area is initiated in the channel formed between layers 2 and 3. This impels the cell onto the injection needle. This flow may optionally be maintained while injection takes place, whereafter the flow is reversed thereby ejecting the cell from the injection needle into the main cell flow. The main cell flow may optionally be paused or slowed while the cell is drawn onto the injection needle, is injected and released. Alternatively, the sequence of flows may be as follows: 1) channel A to channel C until a cell is detected at the injection needle 2) injection 3) channel C to channel B until cell is ejected 4) repeat i.e. go to 1). The length of step 3) will depend on experience of what is required to eject the cell and the desired concentration of the cells coming from the device, it being understood that the device could concentrate or dilute the cells depending on the relative timing of the flow steps.

The relative size and shape of the features and other aspects of the injector device will depend on numerous factors. This will include the type and size of cells being injected, the desired efficiency of injection (i.e. the percentage of cells that are successfully injected), the desired throughput, flow rate, clumping tendency of the cells and other factors.

The device may be fabricated in glass, silicon, plastic or any other suitable material or combination of materials using conventional microfabrication techniques. It will be appreciated that for each material the constraints imposed by the material and manufacturing technique used may require different geometries to those shown in the diagrams contained herein.

We have further found that microfabrication technology offers an approach to optimise the various parameters to suit the cells type and the application. At the design stage, one or more devices are fabricated that have injection areas of different geometries and different arrangements of design elements in a large number of combinations. It is then a straightforward matter to inject cell populations down the different injection areas and determine empirically which arrangement gives satisfactory results according to whatever success criteria are considered important. Furthermore this approach allows the design to be optimised for each type of cell.

Figure 6 - Shows how a number of the devices may be placed in parallel to achieve an even higher throughput. Cells are fed into the microfabricated injectors **1** by a series of splitting channels, one for each injector, and then the resulting injected cells collected from all the injectors in a collecting pool **2**. The material for injection is held in a storage area **4** and pumped through to the injection needle tips by a suitable pump **3**.

It will be clear to the microtechnologist that there are many possible arrangements of the conceptual and material elements of the present invention, and analogues or equivalents thereof, that may be expected to yield a device and method for achieving high throughput injection of cells.

The use of the method and device of the invention are numerous in the fields of, for example, life science research, medical research, drug and agrochemical discovery and diagnostics.

The principle advantage is that the device provides a means of reliably injecting a wide variety of material. Thus it may be used as a generic and predictable transfection method which allows genetic material to be injected into cells with high efficiency. This is of particular advantage when there is a need to transfect cells that are difficult to transfect by conventional means. It is also of advantage when it is required to transfect cells with two or more genes.

The method of the invention may also be used for validating assays used for testing modulation of a biological target. In particular, the situation often arises that the only material that can validate an assay, that is provide a control, is material that cannot be readily provided to the cell interior. The method provides a way of reliably injecting a wide variety of samples.

The device and method of the invention may also be used for validating whether a biological target does or does not influence an important cellular process. In particular the device and method allow for inhibitory antibodies and dominant negative proteins to be incorporated inside the cell in order to ascertain the relevance of target proteins, interactions, enzyme activity and pathways to potential therapeutic intervention. The device and method can therefore be used to prepare cells for a significant number of assays.

The device and method of the invention may also be used for the construction of intracellular assays. In particular, protein and other non-permeable agents or probes, in particular labelled agents or probes may be incorporated into a population of cells that may subsequently be employed for testing and evaluating compounds. In particular labelled antibodies may be injected. Probes may be based on numerous assays principles, for example fluorescence resonance energy transfer (FRET), fluorescence polarisation and fluorescence correlation spectroscopy or may be specifically designed to modulate their signal in the presence of a target molecule or enzyme activity.

The device and method of the invention may be used to evaluate compounds of pharmaceutical or agrochemical interest, especially in cases where there are concerns about the ability of the compound to penetrate the cell. The device allows all such compounds to be reliably incorporated into the cell and reach the site of action.

The device and method of the invention may be used to ascertain the sensitivity of cells to certain compounds which are injected. This will be of value in determining which compound should be employed to treat a particular condition.

The method of the invention may also be used for *ex vivo* therapy, for example *ex vivo* gene therapy. Here a population of cells from a human subject may be removed, microinjected using the device and method of the invention and replaced into the subject.

It will be clear that in many applications it is advantageous to incorporate process or analysis steps on the same device, a micro total analysis system.

Accordingly, we disclose an integrated cell process device which comprises the cell injection function, as described herein, combined with one or more process or analysis steps.

Process steps may include for example further injections or extractions, cell incubation and storage, cell fusion, FACS (fluorescence-activated cell sorting) or other cell sorting, cell lysis, selective cell lysis where certain cells are lysed but not others, or incubation with an agent (a test compound, cell stimulant, nutrient or other) or other environmental factor, such as temprature or light or use in a biological assay. The biological assay may include for example an assay for the biological activity of one or more test compounds. Such an assay may involve incubation of the cell prior to injection or after injection. Optionally the compound(s) may be allowed to contact the cell while it is on the injection needle by for example introducing the compound to the liquid passing the cell: this would allow measurements to be made on the biochemical, physical or electrical response (or any other response) as a result of the compound(s) or other stimuli.

Analysis steps may include flow cytometry, chromatography or analytical analysis of cellular contents, visualisation of cells (microscopy) The process or analysis steps may either precede or follow the injection step, or both. In the case of FACS, this may sort the cells into different channels where they are injected with a different injection material. Subpopulations may optionally be pre-marked for FACS sorting. The applications of FACS and MACS (magnetic-activated cell sorting) has been demonstrated in Telleman P. et al. Proceedings of the μTAS'98 Workshop, Kluwer Academic Publishers 39-.

Process or analysis steps may be incorporated such that they take place either before, during of after the injection process. The sequence of steps will be apparent to the skilled person.

A further application of the invention is that it may be used as a means of extracting material from inside cells. This may be achieved by simple reversing the flow in the injection channel. This ability may be exploited either to harvest an intracellular cell product, which may be for example a protein or genetic material, or it may be used to sample cell contents for subsequent analysis.

Figure 8 - Is a diagram of a microfabricated cell injector with a conduit consisting of three parallel channels defined between 4 layers (**Layers 1 to 4**). Cells pass in the direction A to B in the channel defined by **layer 1 and 2**. An oscillating pressure is applied in the second channel **2** which impels a passing cell onto the injection needle **1** and in the opposite direction forces the cell off the injection needle and back into the flow of A to B. Alternatively the flow is from A to C until a cell is detected at the needle and then, after injection, the flow is from C to B for a period. The third and fourth layer define the internal surface of the injection needle and carries the material for injection into the cells. The injection needle has a height in the order of 3 micron above the upper surface of layer 2, the walls are approximately 1 micron thick and the internal diameter of the needle is 1 micron at its narrowest point.

In an alternative feature of the invention material is not injected into the cell but is extracted, such as parts of the cytoplasm or organelles (e.g. the nucleus or mitochondria). This alternative feature of the invention may allow for several new uses which previously have not been viable, except on a very small scale. These uses include the following:

1. <u>Analysis of cell contents</u>- a sample of cytoplasm, or an organelle of the cell(s) may be extracted and analysed to analyse the content of the cell (analysis of, for example, specific proteins, the proteome, metabolites or nucleic acids). This analysis may be performed before or after exposure of the cell(s) to a compound or environmental factor. Such a compound or environmental factor (such as a cytokine) may, optionally, be delivered into the cell directly by the needle prior to extraction.

2. Measuring Cell Permeation - An important factor in pharmaceutical development is to determine the availability of the compound to its biological target within the cell. By extracting samples directly from the cells direct measurements of the presence of the compound may be made. It is possible to determine the distribution of the compound within the organelles or cellular compartments of the cell. Alternatively the entire contents of a cell may be vacated and replaced by buffer solution. These empty cell sacs may be used to measure whether compounds diffuse across cellular membranes, or are actively transported, and the rate of diffusion/active transport.

3. In-cell PCR - Cell microinjection allows access to the cell interior for in-cell PCR .

The device is also able to be used to measure the electrical properties of cells for example by means of electrodes placed in the injection channel and the channel containing the cells. For example, the membrane potential and membrane permeability may be measured on populations of cells, optionally in response to external agents or test compounds.

**Construction of the Devices**

Microfabricated devices of the invention may be prepared by standard techniques currently employed by microtechnologists. By way of example the following suggested route for manufacture of the device in Figure 8 is provided.

The injection needle has a height in the order of 3 micron above the upper surface of layer 2, the walls are approximately 1 micron thick and the internal diameter of the needle is 1 micron at its narrowest point.

Several methods for fabrication of the needle could be devised by a skilled technologist. For example, layer 2 could be fabricated from glass or silicon by 1) patterning to give cylindrical resist cores in the desired positions on the chip, 2) sputtering with glass or metal over the cores at an angle away from normal to cover the sidewalls, 3) planarising with resist and ion milling off the top to leave a core surrounded by an open cylinder of glass or metal, 4) removing the resist to leave a cylinder and 5) anisotropically etching from the rear to

connect with the hole in the centre of the cylinder. It will be understood that other features such as channel walls, spacers, electrodes etc. will also be incorporated during the fabrication of the needle feature.

Various techniques may be employed to sharpen the tip of the injection needle to aid penetration. For example milling techniques may be employed. Alternatively, the 5-step method described above may be elaborated by the provision of a thin metal disc, of diameter slightly greater than the resist cores, positioned centrally on top of the resist cores. The sputter coating to form the sides of the cylinder is then carried out from above at an angle such that the overlap casts a "shadow" on the sides of the resist, giving a sloping internal surface with the wall tapering to the top. The metal disc and resist are then removed and the etch from the rear step carried out as before. Connections with liquid resevoirs external to the device may be made in accordance with Mourlas N.J. et al. Proceedings of the µTAS'98 Workshop, Kluwer Academic Publishers 27-, and references cited therein.

Example 3 Figure 10 - Shows one of several methods that can be used to produce micro-needle structures. This particular method results in needles made of silicon nitride.

1. Deposit a silicon nitride masking layer onto a silicon wafer
2. Pattern masking layer using photolithography and plasma etching to expose areas of silicon.
3. Etch silicon using $HF/HNO_3$ isotropic etch to form points of appropriate dimensions
4. Remove silicon nitride layer
5. Deposit silicon nitride
6. Pattern masking layer on back of wafer
7. Apply a thick photo-resist layer to form to the wafer to coat most of the points
8. Remove the exposed region of silicon nitride
9. Remove the photo-resist
10. Etch the silicon using KOH anisotropic etch to form hollow needle structures

In a continuation of the the above process to produce a housing to surround the needle- figure10a:

11. Deposit strike layer for electroplating over the front side, resist on front side, pattern to form openings where sacrificial layer will be electroplated to form suction channel

12. Plate up sacrificial layer (Cu or Ni) to thickness of suction channel

13. Apply SU8 layer #1 to a thickness equal to the desired depth of the trap well

14. Expose SU8 #1 - hardens in exposed areas - areas of trap well and vias from suction channels to suction inlets are masked but not developed away.

Referring to fig. 10b:

15. Apply SU8 layer #2 to define cell inlet and suction inlet. Expose to pattern channels

Referring to fig. 10c:

16. Develop both layers of SU8

17. Etch the silicon from the rear side only in KOH to open the cell inlet channel via to the needle, and make the needle hollow. The front side should be protected during this process. The sacrificial layer is left in place to protect the Au / SU8 interface from the KOH.

18. Remove sacrificial layer to open suction channel

18 Seal transparent top cap to structure to close cell and suction inlets

19 Seal rear cover layer to form injection channel inlets.

**1 Down-hole sputtered needle process**

1 Start with dissolvable substrate (copper sheet, flattened) - figure 11a(1)

2 Spin on SU8 to depth for wells (20-30 micron).

3 Expose using **mask SU8-1b** and develop to produce wells and openings for via to "suck" channel connection - figure 11a(2)

4 Plate up copper to part fill well so that depth sets needle tip position - figure 11a(3)

5 Spin on positive resist (thick enough to form suck channel over flat areas - around 3 microns, but considerably deeper in well).

6 Expose using **Cu Mask** so that "needle hole" is near centre of well. Alignment to ~5 micron is acceptable.

7 Develop. Heat to soften resist and round edges - figure 11a(4)

8 Expose to copper etch sufficient to remove ~1-5 micron of copper at openings in resist to produce undercut.

9 Sputter on needle wall forming film e.g. SiO2 or glass to ~1 micron on level areas figure 11a(6)

10 Spin Su8 (~10 to 30 micron) and expose using **Cap Mask** aligned so that cap features are directly above needle holes. Alignment accuracy better than ~7 micron is acceptable.

11 Develop to open port behind injection needle structure -figure 11a(5)

12 Take glass sheet with sawn channel ( 0.5 to 5 mm wide) for injection supply and align and stick this onto coated substrate using adhesive (e.g. epoxy, UV cure acrylic, 3M self adhesive films) -figure 11b(7)

13 Dissolve original (copper) substrate.

14 Inverting to use glass sheet as lower level of substrate, spin on SU8 (~50 micron) and expose using **mask SU8 2 with reversed symmetry** to form cell feed and solution suck channels. Alignment to 10 micron is acceptable.

15 Cap with glass sheet coated with adhesive (e.g. as above in 12). Glass sheet should have pre-cut or etched channels to allow connection of tubes - figure 11b(8)

**Horizontal Needle Method**

1 All over one surface of flat transparent substrate (glass, Silica or possibly polymer) deposit opaque metal insoluble in Copper etch (e.g. Ni, Al, Cr).

2 Spin on positive resist (thin), and expose using **mask SU8 1b.** Develop pattern and etch the metal - figure 12a(1)

3 All over substrate deposit/ plate copper to thickness required for suck channel (~3 micron).

4 Spin on positive resist. Expose using **Cu Mask.** Align so that needle point hole in Cu is in centre of metal circle corresponding to well. Alignment to 5 micron is acceptable. Develop. Etch copper to form rectangles - figure 12a(2)

5 Remove positive resist and spin on SU8 to ~ half depth required for wells (10-15 micron).

6 Expose using **Mask SU8 1b** so that wells align with "needle position hole" in Cu, and so via for suck channel is over other end of Cu rectangle. Alignment not critical, but should be not more than 10 micron misalignment. Leave undeveloped see figure 12a(3)

7 Coat all over with sacrificial metal (Ag, Cu, Zn) to ~1 to 3 micron.

8 Coat all over with needle core metal (Al, Ni) (# 1-3 micron).

9 Spin on positive resist. Expose with mask having narrow (1 - 3 micron) dark bars. This can probably be on **Cap Mask.** Thin bar should be aligned to centre of well ("needle position hole") so that bar extends over half way across well. Alignment to ~5 micron may be required - figure 12a(4)

10 Needle core metal is etched to form bar without etching the sacrificial metal.

11.Positive resist is removed. Positive resist is spun on and exposed using **Mask SU8 1b** displaced from previous position by amount which defines projection length of final needle (3 - 15 micron). The resist is developed - figure 12a(5)

12 The sacrificial metal layer is etched without etching the needle core metal leaving the needle core metal bar as a bridge with ends covered with positive resist.

13 The needle core metal is treated by oxidation, anodisation, or plating to form an insoluble coating on the exposed surfaces -figure 12a(6)

14 The substrate is coated with SU8 to a thickness to form the top half of the well structure (10-15 micron), and exposed using **mask SU8 1b** Alignment of well centre probably needs to be to 5 micron or better, depending on how long needle projection is set. Also invert substrate and expose without mask through substrate to harden SU8 under part of bar - figure12b(7)

15 Spin on SU8 to thickness for feed channels (~50 micron) and expose using **Mask SU8 2.** Alignment error of up to 10 micron is acceptable figure 12b(8)

16 Develop through three SU8 layers - figure 12b(9)

17 Dissolve/ etch positive resist, sacrificial metal in openings, and copper between well and needle suck via third well- figure12c(10)

18 Dissolve out needle core metal. Dissolve out opaque metal films on substrate if required.

19 Stick adhesive coated glass sheet on top to enclose channels. Sheet should be pre-cut or etched to form entrances for attaching tubes - figure 12c(11).

Figure 13 is a photograph of the device of figure 14

Fig. 14 shows a plan view of a cell injection device fabricated according to the method of example 3 in which a device has 4 injection positions 12 - 18 at which cells are to be injected. Although four injection positions and associated channels are shown it is understood that the general features of the device in fig. 1 are capable of extension to more than 4 positions with suitable layout of the device. Each injection position has an inlet channel 20 and an outlet channel 22. Also provided is a suction channel 32 - 38 which communicates with a trap well (not shown) at each injection position, which trap well acts to channel flow from the inlet channel to the suction channel, thereby directing cells in the inlet channel in the vicinity of the injection position into the trap well. Positioned within the trap well is an injection needle (not shown), which injects the cells when they reach the correct position in the trap well. The suction channels communicate also with a suction port 40, 42, 44, through which flow can be established in either direction. When the cell is to be trapped and injected, flow is directed from the inlet channel towards the suction port; when the cell is to be ejected from the trap well, the flow is reversed. The injection needles communicate with an injection channel through which material flows to and/or from the needle. In the embodiment in fig. 1 the injection channel is common to all four needles, though separate injection channels might be provided. The injection channel is in the form of a via through the thickness of the device; the outline of the injection via on the rear of the device is shown at 48. The via communicates with an injection port on the rear of the device which carries injection material to and/or from the via. In the embodiment in fig. 1 the suction channels 32 and 38 are shown each to communicate with one suction port; suction channels 34 and 36 communicate with a common port 42. Variations of connections of the inlet, outlet and suction ports are possible where a variety of degrees of commonality between them are envisaged. In the embodiment in fig. 1 the inlet channels communicate with the branching 'binary division' arrangement 50, which serves to divide a flow of cells entering from the left in fig. 14 into four statistically even flows, one through each of the inlet channels 20. The inlet and outlet and suction ports are terminated by broadened sections 52 into which capillary connections may be sealed allowing connection to flow systems off the device.

Dimensions of the device are chosen to suit the type of cell or other object to be injected but will typically be between 5 and 200 um across for the inlet and outlet channels and the suction port. The trap well diameter will be between 1x and 2x the diameter of the object to be

injected. If the object is deformable the trap well diameter may be less than the diameter of the object. The suction channel dimensions will be such as to prevent passage of the object from the trap well into the suction channel and so at least one dimension of the suction channel will be less than the minimum dimension of the object; substantially less in the case that the object is deformable. In the embodiment shown in fig. 1 the inlet and outlet channels are 40 um wide by 25 um deep; the suction port is 100 um wide by 25 um deep; the trap well is 25 um diameter by 25 um deep and the suction channel is 150 um wide by 3 um deep. These dimensions are optimised to trap and inject substantially spherical objects between 12 and 25 um diameter.

Fig. 15 shows a more detailed view of one of the injection positions 12 in an injection device of the invention, with the same nomenclature as fig. 14. The trap well 60 is shown with the injection needle 62 in the base of the well. The fluid flow paths in the device are from inlet channel 20 to outlet channel 22 to introduce objects to be injected into the device; from the inlet channel down through trap well 60, surrounding needle 62, through suction channel 32 and up through via 64 into suction port 40. Supports 66 are provided to hold the structure defining the floor of the inlet channel 22 above the roof of the suction channel 32 above the floor of the suction channel, while allowing unimpeded flow through the suction channel.

Fig. 16 shows an overall plan view of a chip comprising several injection devices of the invention of different configurations. Chip 10 has on it injection devices 100 - 106, each separately operable and intended to demonstrate various possible configurations of serial and parallel operation of injection. Device 100 is as shown in fig. 14. Device 102 has five injection positions in series with common inlet and outlet channels, common suction port and common injection channel. Device 104 has a single injection position and two suction ports to give even and symmetrical suction flow around the object to be injected in the vicinity of the trap well. Device 106 has a single injection position and a single suction port, and is as shown in greater detail in fig. 15. The additional structure 110 shown as part of device 106 is a dummy channel which might be used to communicate with the trap well for purposes such as addition of material into the well in the intimate vicinity of the cell, for example to test cell responses to compounds while in-situ on the needle.

The devices shown in figs. 14-16 are all operated by external sources of fluids and driving force, for instance pressure and/or vacuum. Control means (not shown) for controlling such driving forces might be external to the devices are integrated wholly or partially with them; for instance micropumps might be integrated onto the device. It is understood that electrodes might be integrated onto the devices in ways known in the art, in order to provide either driving force for fluids, e.g. by electro-osmotic flow, or for cells or other objects, e.g. by dielectrophoresis, or to provide sensing of the position or electrical potentials associated with cell physiology or other properties of objects. The electrodes might also provide sensing or control functions for fluids surrounding the cells or objects.

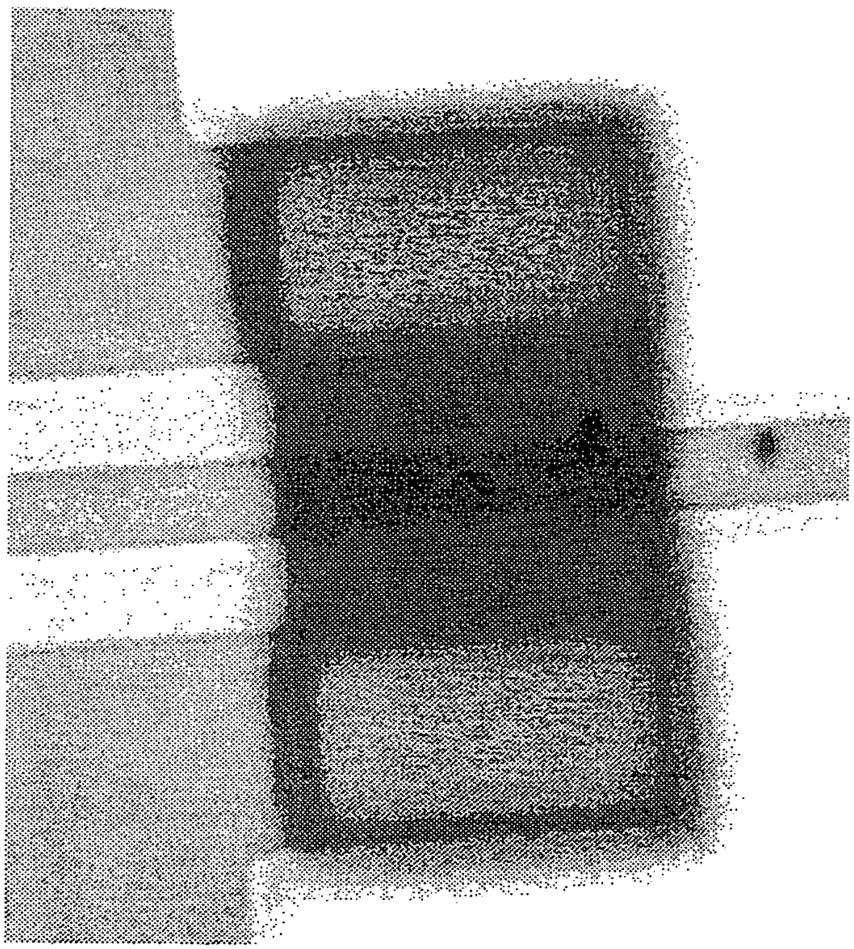
The picture below AA shows a close-up view of an injection device as shown diagrammatically as item 104 in fig. 16, fabricated by the integrated process [example 3 above] in SU8 photopatternable epoxy on an Si chip. The inlet and outlet channels are shown with the trap well leading down from them into the device. Two suction ports are provided, one each side of the inlet and outlet channels. The injection needle is situated at the bottom of the trap well and appears as a small light dot. The small size of the needle (12 um in height, 2 um across at the top) makes it hard to image in the depth of the trap well, which is 50 um deep from the top surface of the device, 25 um deep below the floor of the inlet and outlet channels. The microscope is focused at the level of the top of the needle, so the details of the suction channels are indistinct.

Other dimensions of the parts of the image are: inlet and outlet channels, 40 um wide by 25 um deep; trap well 25 um diameter by 25 um deep above the base of the needle; needle: 12 um high above the base, 2 um diameter, 0.1 um needle material thickness, as shown in fig. 9 [in patent document 99-107 at present]. Suction channel: 4 um high above the base of the needle, 150 um wide.

Figure 13 - Shows a general view of an injection device as shown diagrammatically in fig. 14 and as item 100 in fig. 16, fabricated by the integrated process as for Pciture AA below. The inlet channels to the four injection positions are fed from a common inlet port via a binary division tree; the outlets go to separate ports in this design. The features of the device are as

described for fig. 14. Dimensions of the various parts are as above. Slight cracks are visible at the corners of some of the channels. these arise from stress in the SU8 layers used to form the microchannels and do not affect the working of the device or the integrity of the structure.

Picture AA



## Claims

1. A microfabricated cell injector comprising an injection wall and projecting from the injection wall a cell injection needle, such that in use cells suspended in a fluid are impelled towards the injection wall and pierced by the injection needle whereupon material is (1) injected into the cell, (2) extracted from the cell, or (3) injected into the cell and then extracted from the cell the steps being in any order and any number of times.

2. A microfabricated cell injector as claimed in claim 1 further comprising cell propulsion means for impelling cells towards the needle.

3. A microfabricated cell injector as claimed in claim 1 or 2 wherein the needle is held within a housing defined by the internal surfaces of the microfabricated cell injector, the housing having an inlet for suspended cells to enter and an outlet for cells to exit.

4. A microfabricated cell injector as claimed in claim 3 wherein a single inlet for cells to enter the housing is also the outlet for cells to exit the housing.

5. A microfabricated cell injector as claimed in claim 3 wherein the microfabricated cell injector has a number of housings each with at least one needle wherein suspended cells for injection are divided and each divided stream of suspended cells is fed through one housing.

6. A microfabricated cell injector comprising an internal surface defining a conduit, which in use transports cells suspended in a fluid, the conduit having an inlet and an outlet, the conduit further comprising a cell injection needle, such that, in use cells enter the injector via the inlet, are moved along the conduit and are pierced by the cell injection needle whereupon material is (1) injected into the cell, (2) extracted from the cell, or (3) injected into the cell and then extracted from the cell the steps being in any order and any number of times, and the cells are then moved to the outlet.

7. A microfabricated cell injector as claimed in any of the above claims where the needle is a hollow structure and injection or extraction is actuated by a cell sensor which determines the presence of a cell on or nearby the needle.

8. A microfabricated cell injector as claimed in any of the above claims which additionally comprises a cell capture sensor which determines the presence of a pierced cell on the injection needle and actuates injection of material into the cell or extraction of material from the cell.

9. A microfabricated cell injector as claimed in claim 8 wherein the cell capture sensor prevents further cells being impelled towards the needle.

10. A microfabricated cell injector as claimed in claim 8 or 9 wherein the cell capture sensor actuates the expulsion of the cell from the needle after injection of the material into the cell or extraction of material from the cell.

11. A microfabricated cell injector as claimed in any of the claims 1 to 6 wherein the needle is solid and material for injection is present within the fluid suspending the cells.

12. A microfabricated cell injector as claimed in any claim from 1 to 6 wherein the needle is a non-cell piercing hollow structure and cell piercing is achieved by the application of a cell disrupting chemical or force through the end of the non-cell piercing needle structure.

13. A microfabricated device containing a plurality of cell injector units as claimed in any of the above claims wherein the respective inlets and outlets of the cell injecting units being each connected such that the cells are divided into each injector unit and recombined after injection.

14. A method for the microinjection of cells which method comprises passing a suspension of cells in a fluid through a conduit comprising a cell injection needle, the cells thereby being pierced by the injection needle and material is: (1) injected into the cell (2)

extracted from the cell or (3) injected into the cell and then extracted from the cell the steps being in any order and any number of times; as the cells pass through the conduit.

15. A method for the microinjection of cells which method comprises passing a suspension of cells in a fluid through a device as claimed in any one of claims 1 to 13.

16. Use of a needle which is hollow and substantially circular in cross section, the external diameter of the needle continuously decreasing as it projects from the base of the needle to its tip, the tip being less than 25 microns, preferably less than 5 microns, in diameter in the piercing and injecting of material into, or extracting material from, cells.

17. An integrated cell process device which comprises a microfabricated device as claimed in any claim from 1 to 13 combined with one or more process or analysis steps

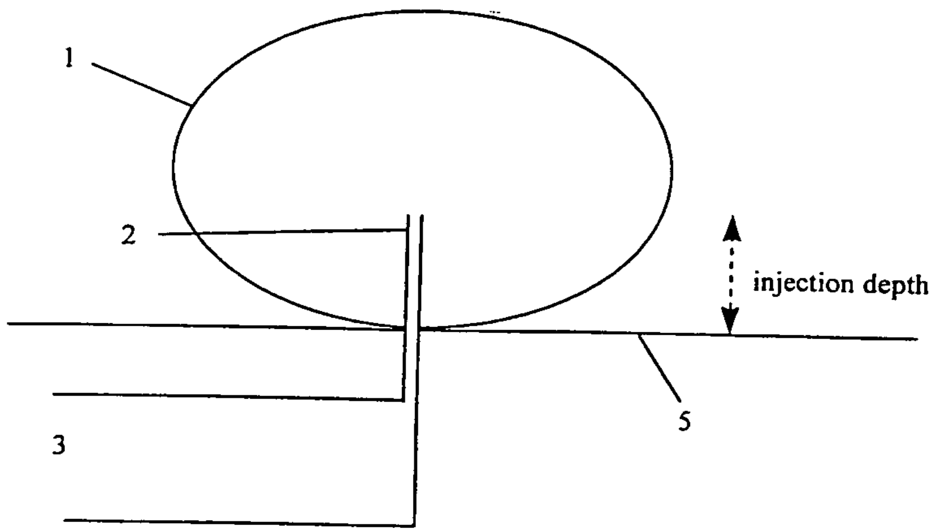



Fig. 1

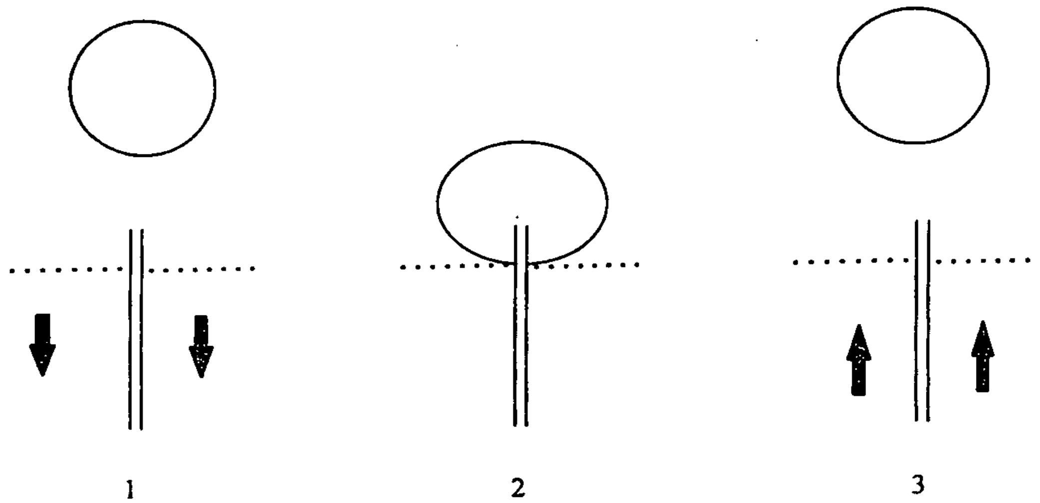



Fig. 2

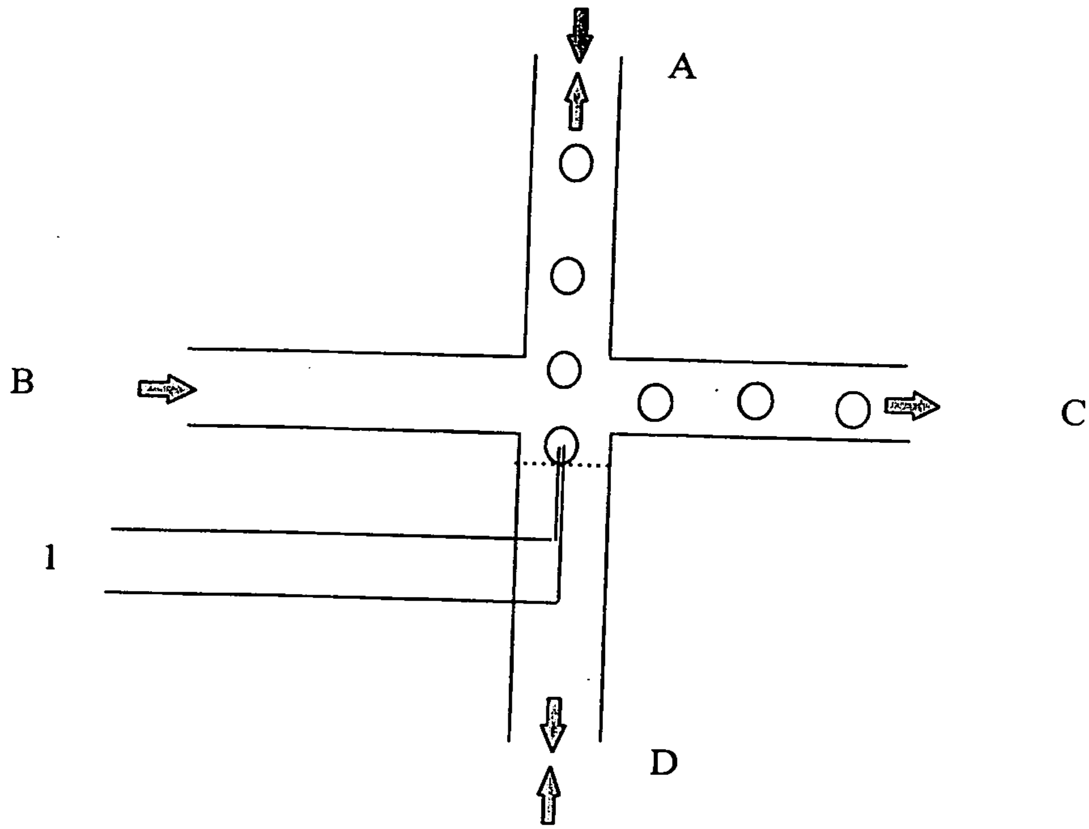



Fig. 3

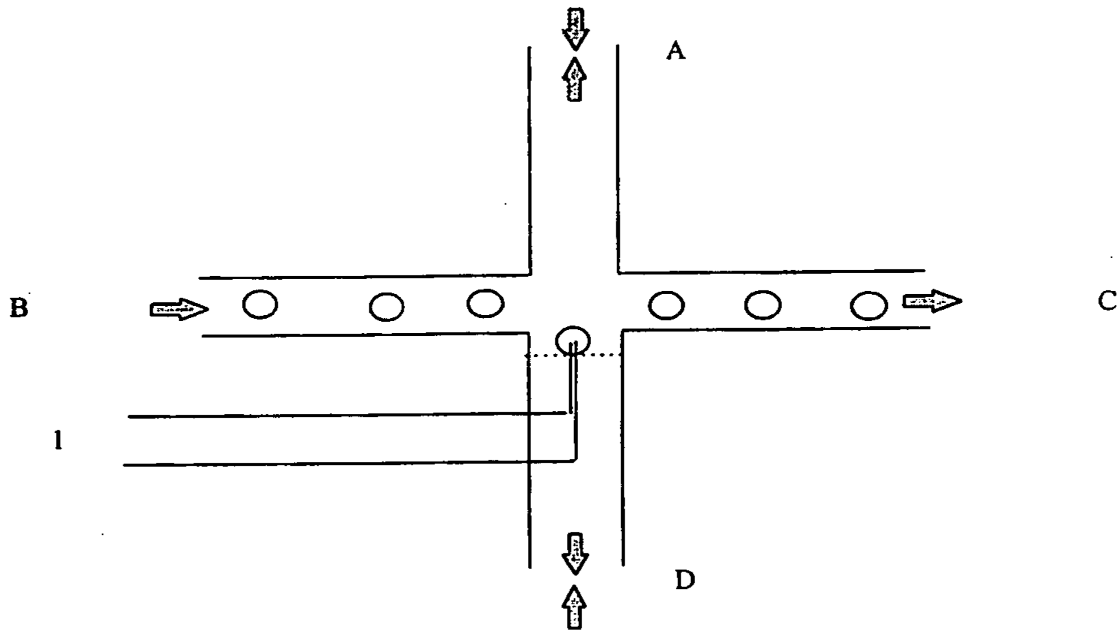



Fig. 4

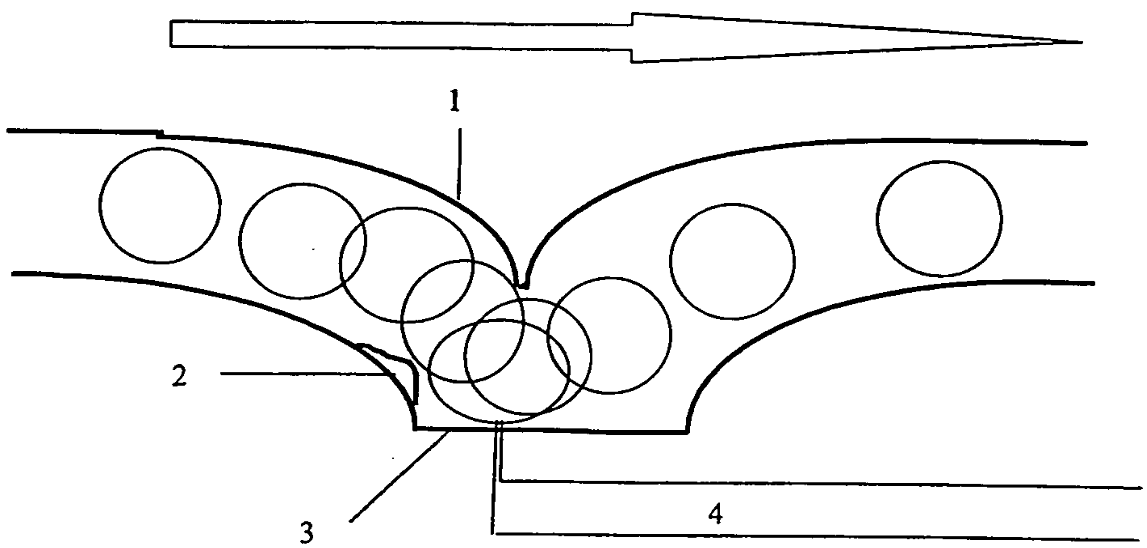
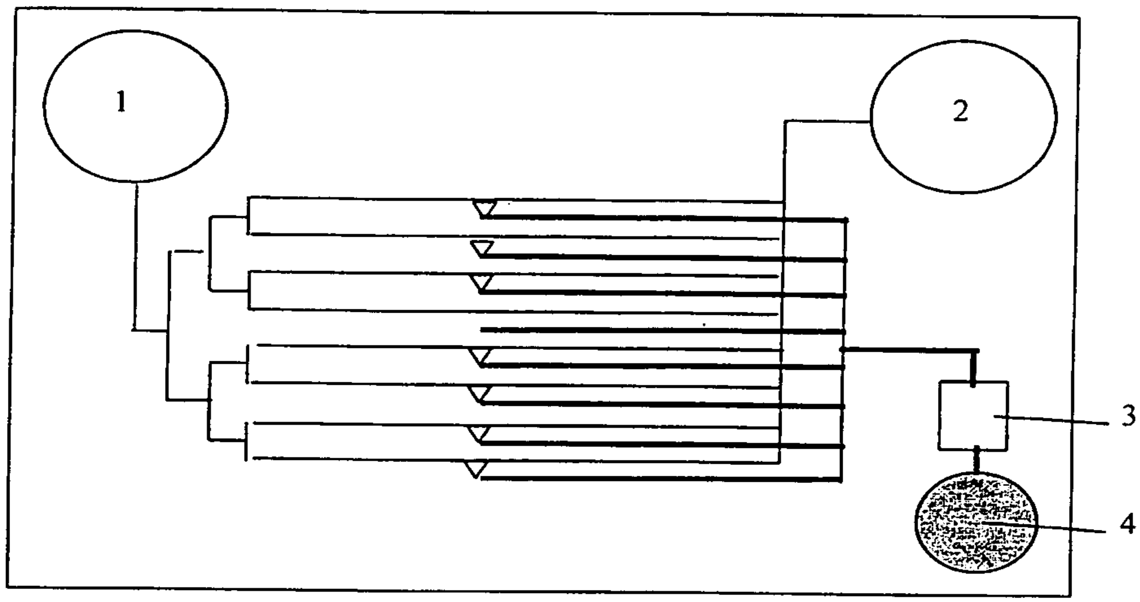



Fig. 5




▽ = injection area

Fig. 6

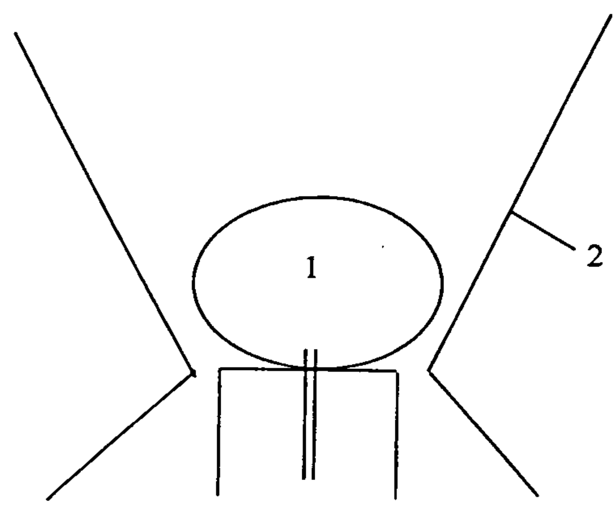



Fig. 7

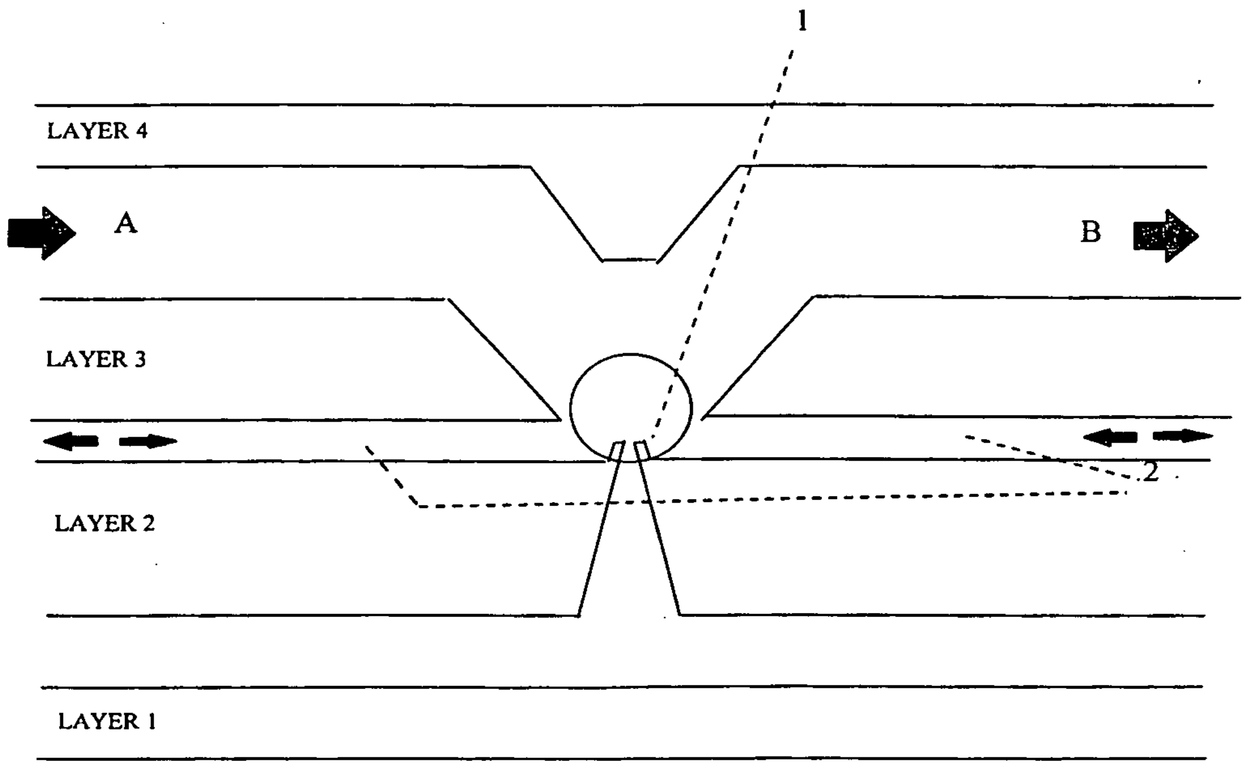



Fig. 8

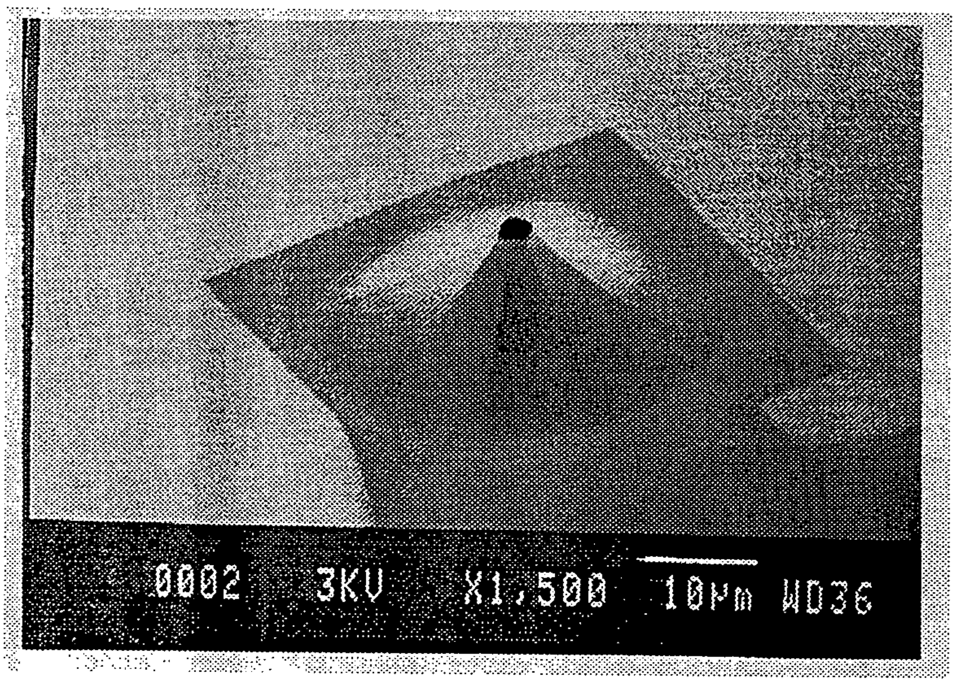



Fig.9

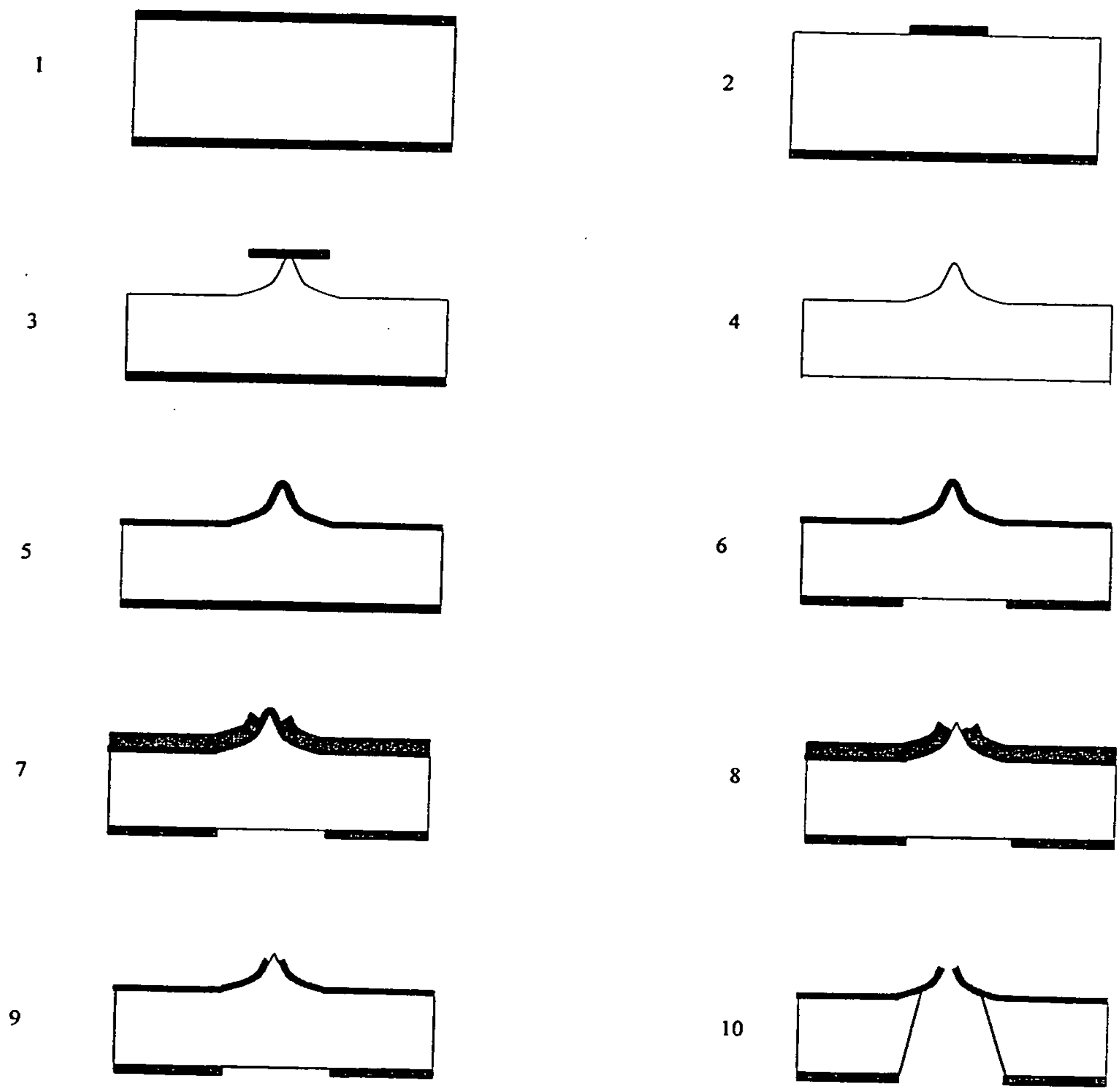



Fig.10

Fig 10a

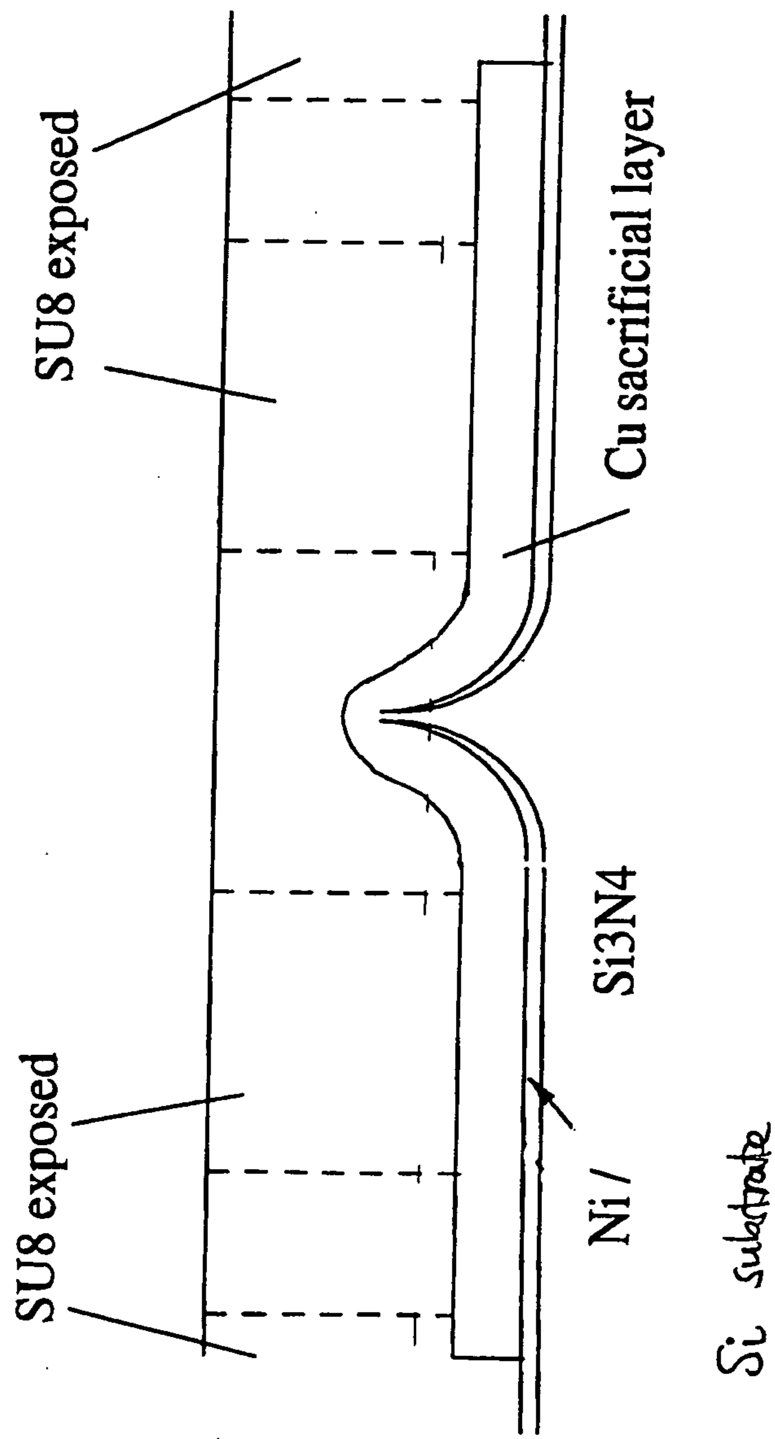

SU8 exposed
Cu sacrificial layer
Si3N4
Ni /
SU8 exposed
Si substrate

Fig10b

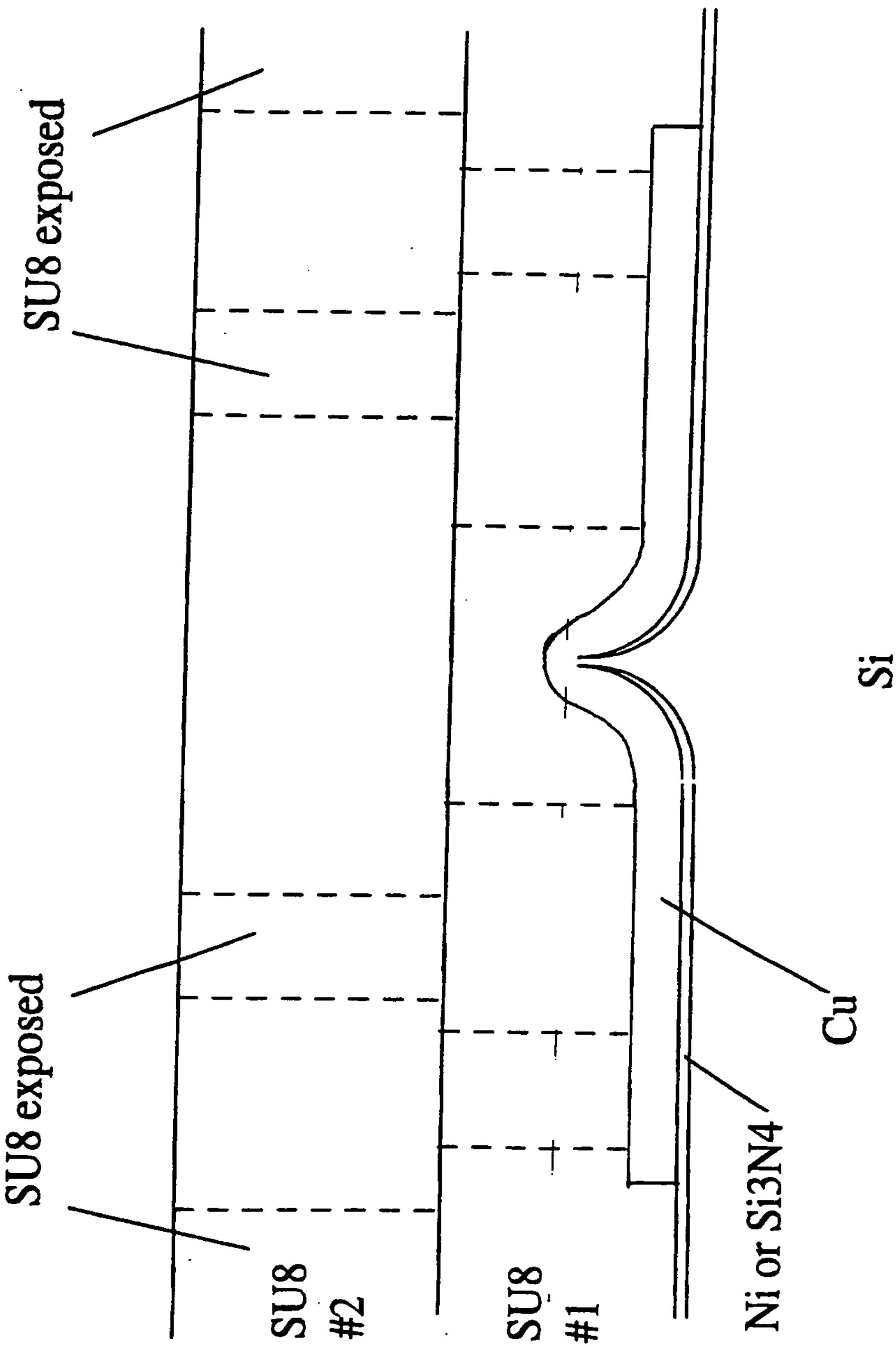
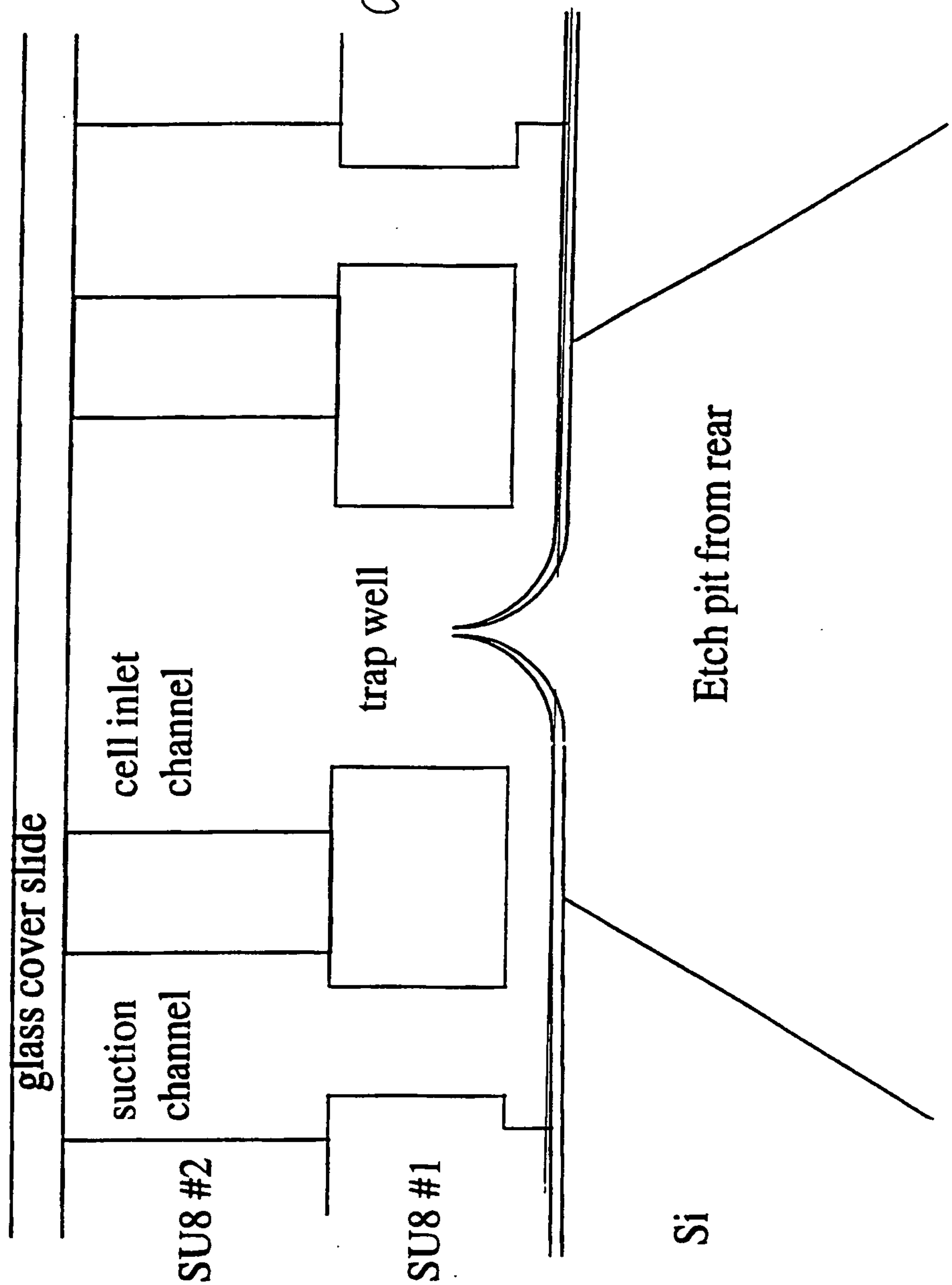
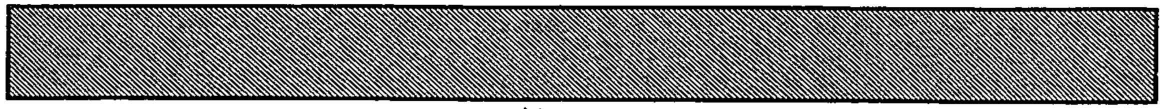
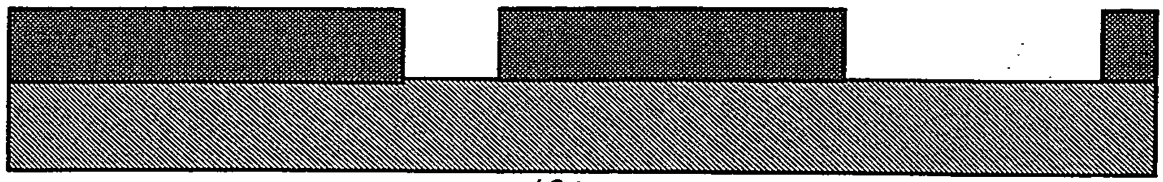


Fig 10c

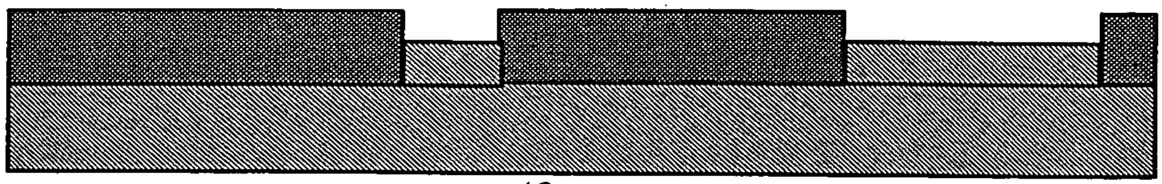

glass cover slide
suction channel
cell inlet channel
trap well
Etch pit from rear
SU8 #2
SU8 #1
Si



(1)



(2)



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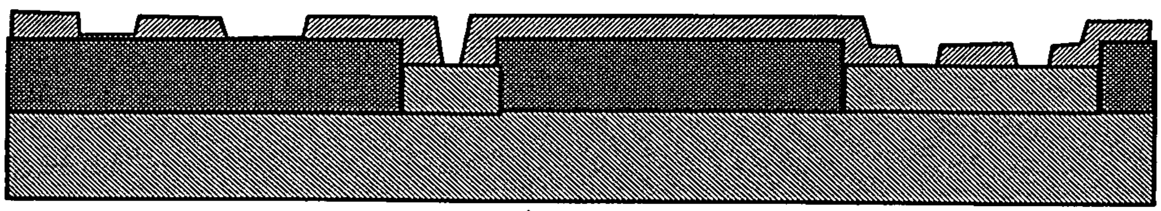

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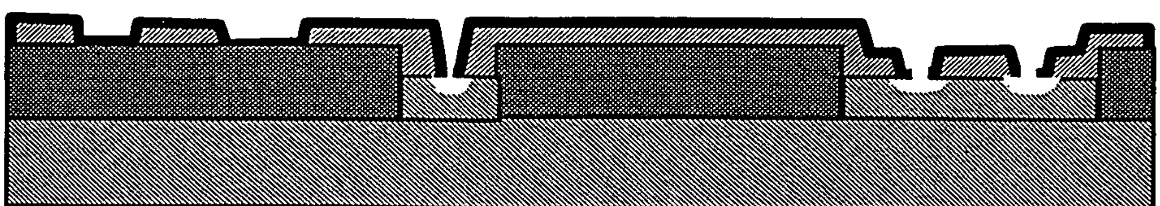

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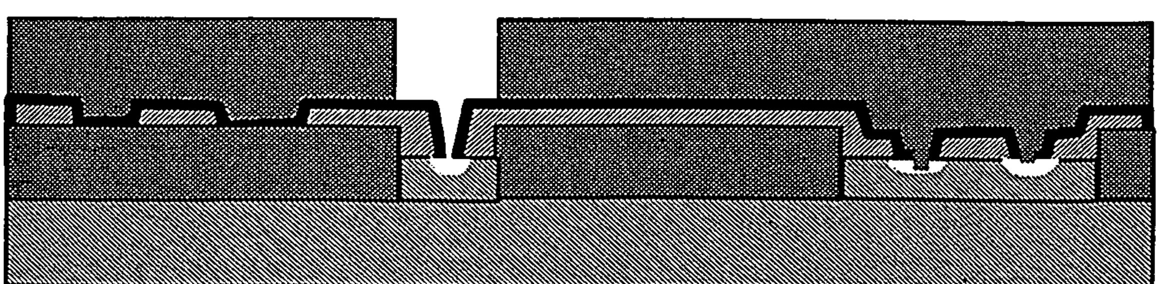
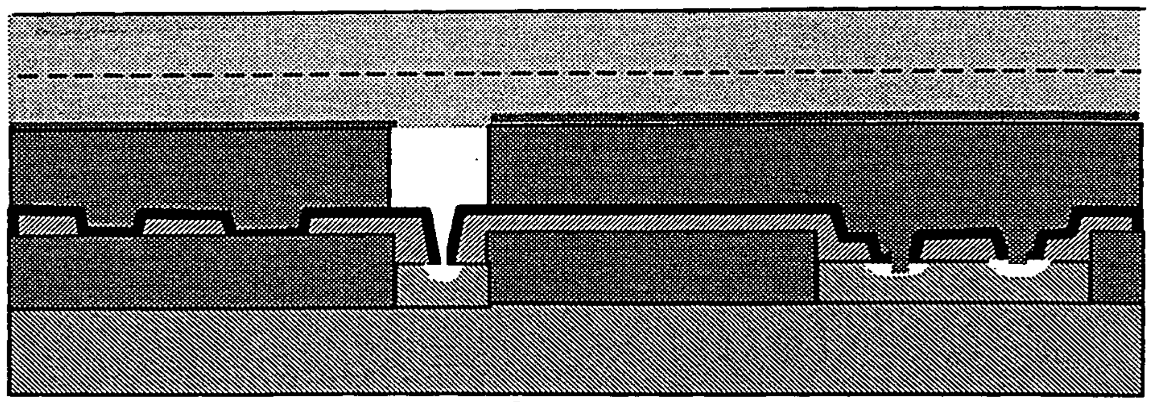


Fig 11a (6)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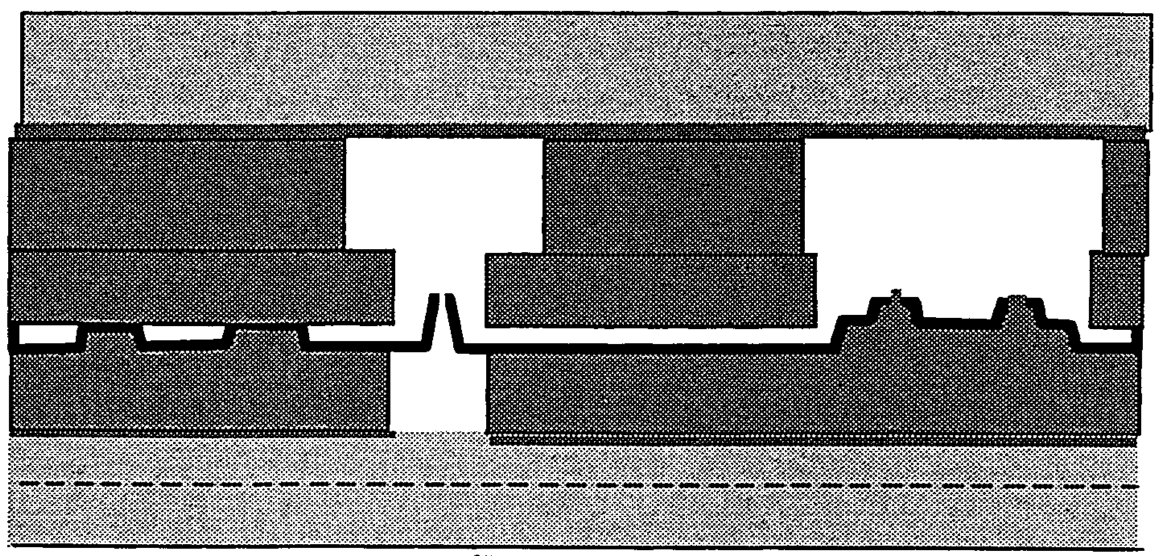
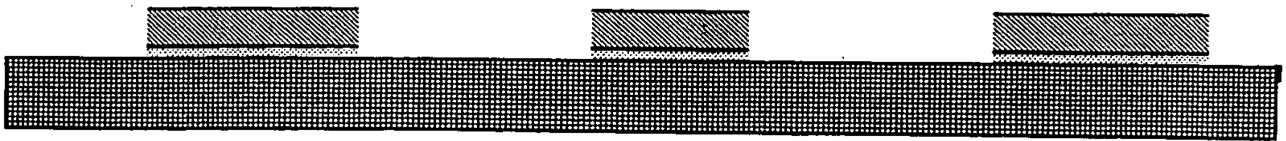

(8)

Fig 11b



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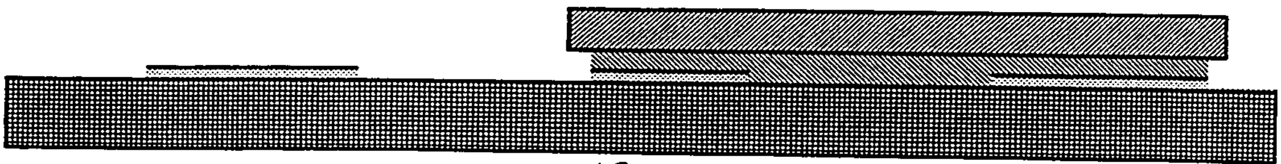

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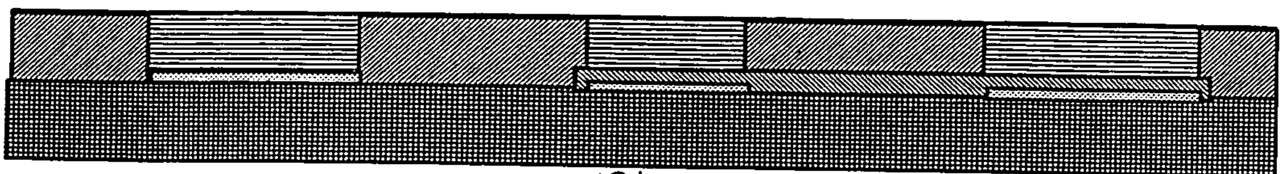

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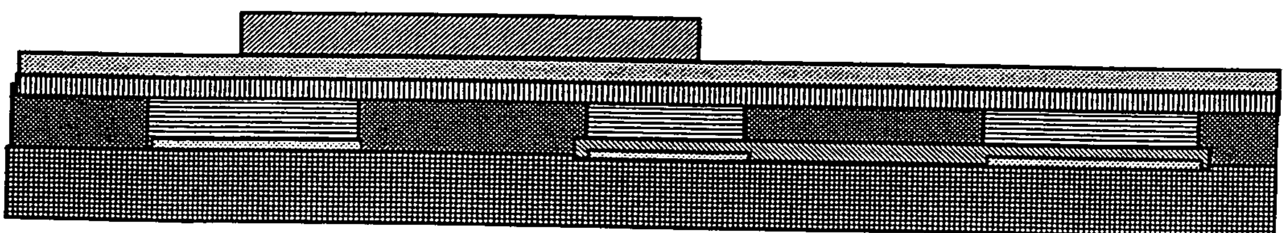

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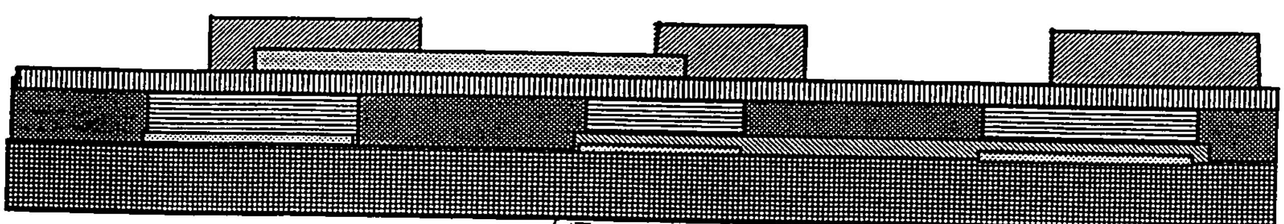

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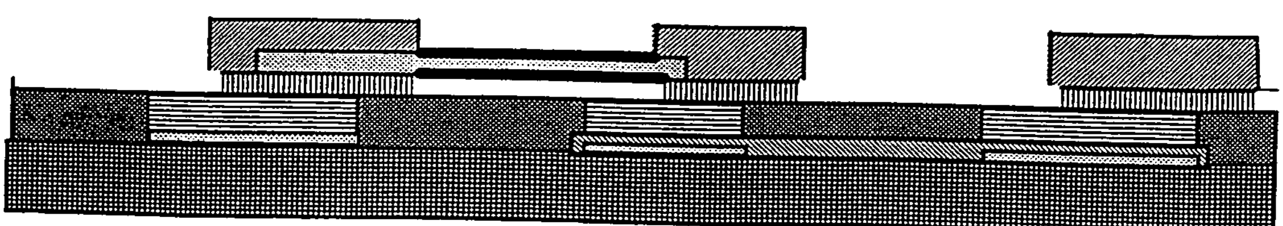


(6)

Fig 12a

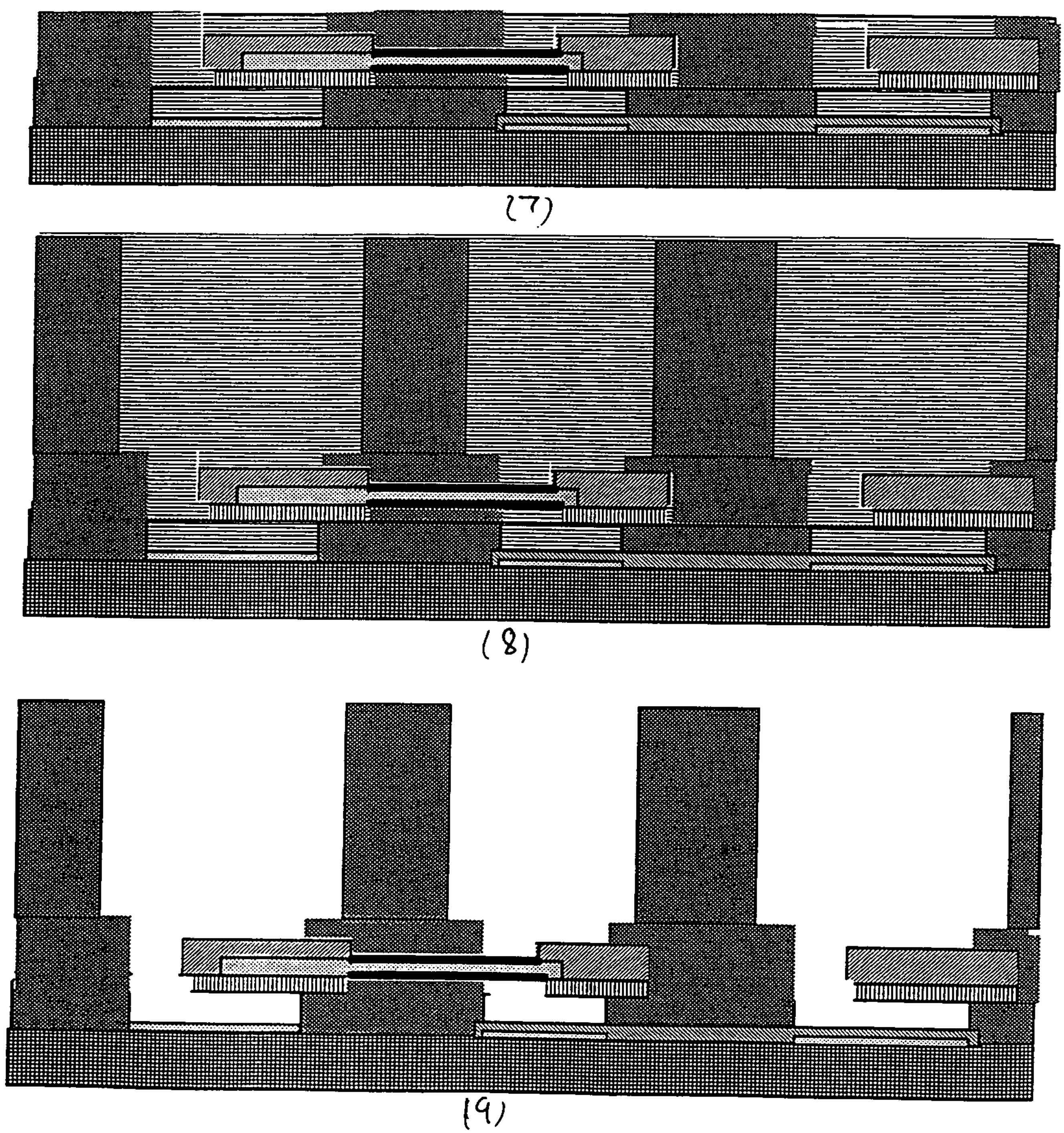
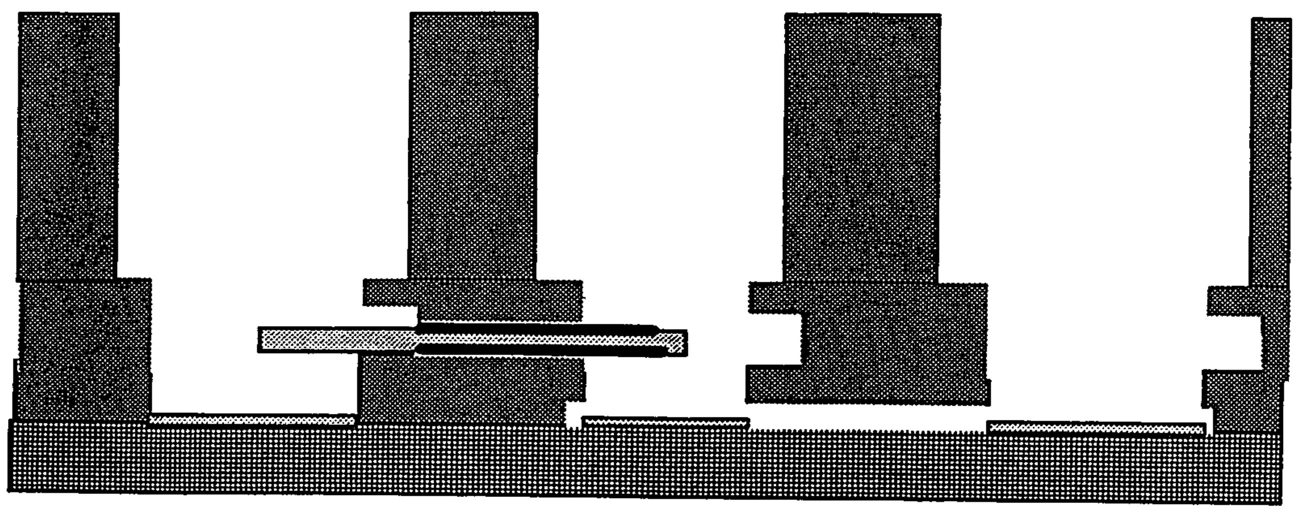
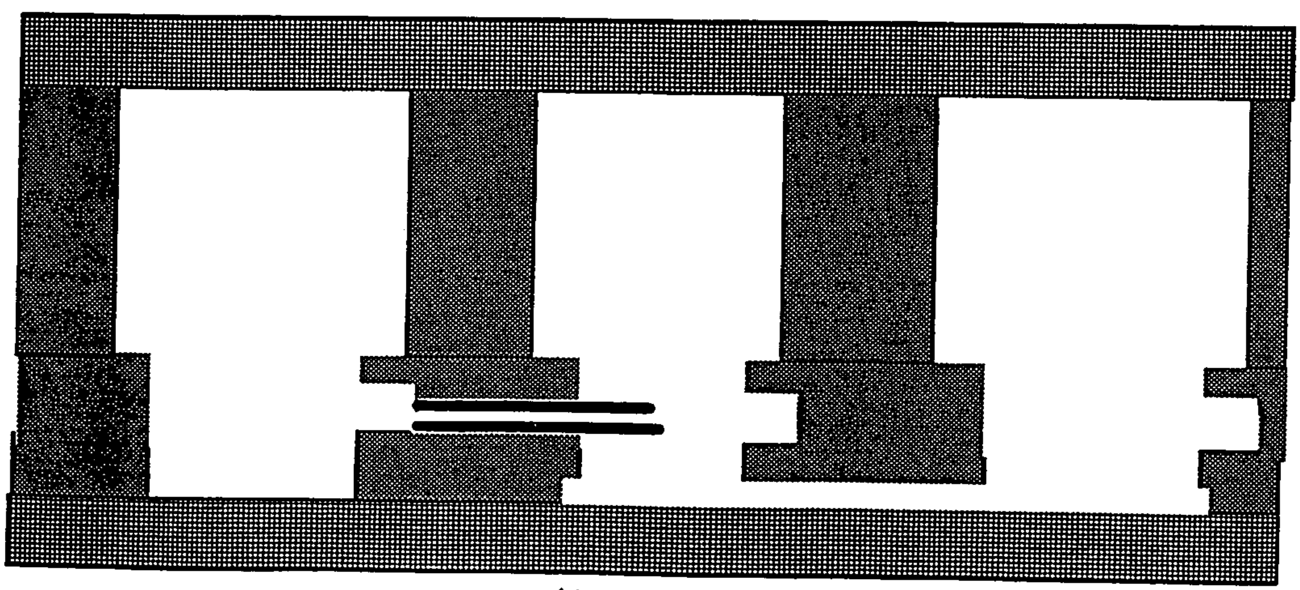



Fig 12b



(10)



(11)

Fig 12c

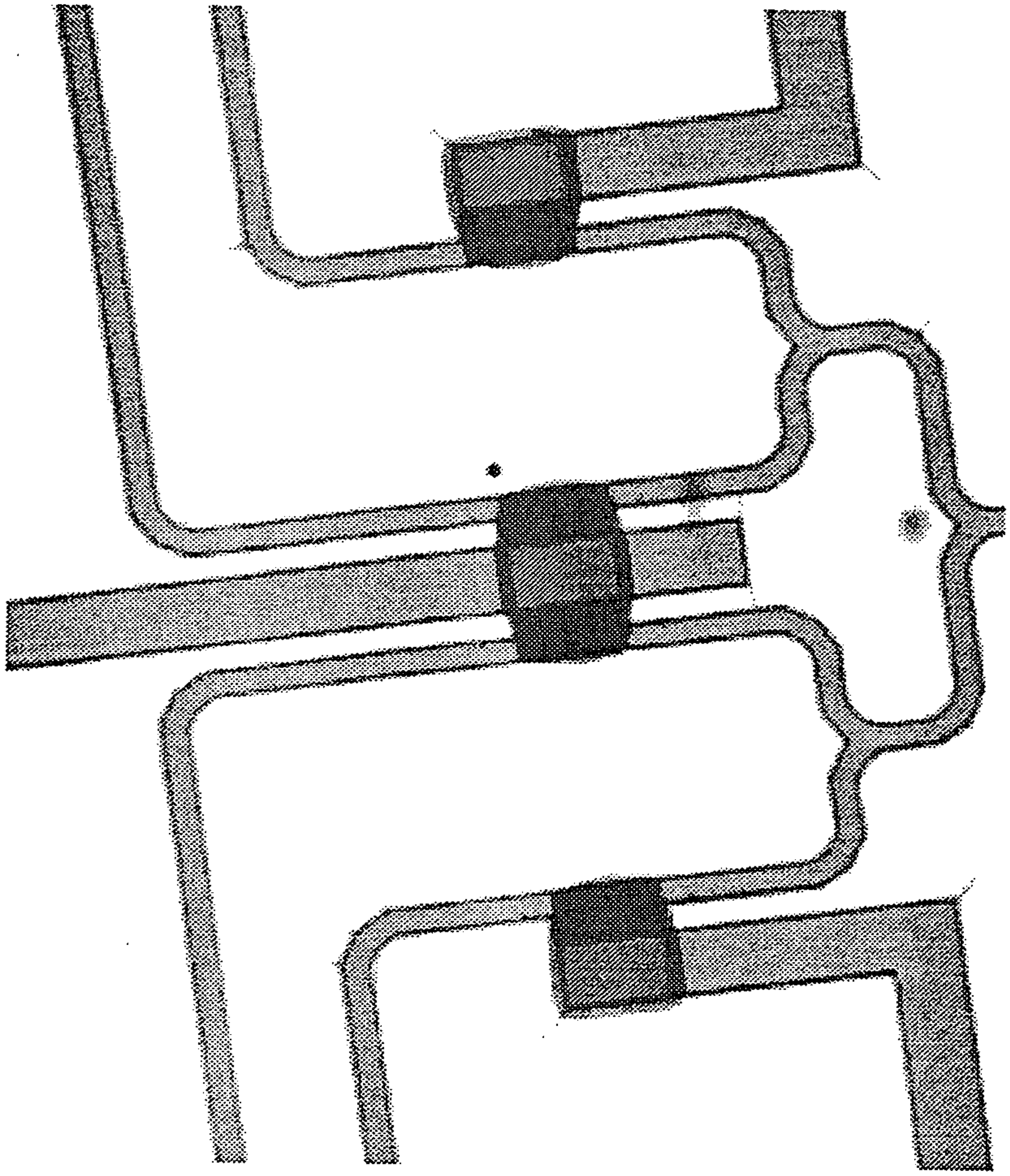


Fig 13

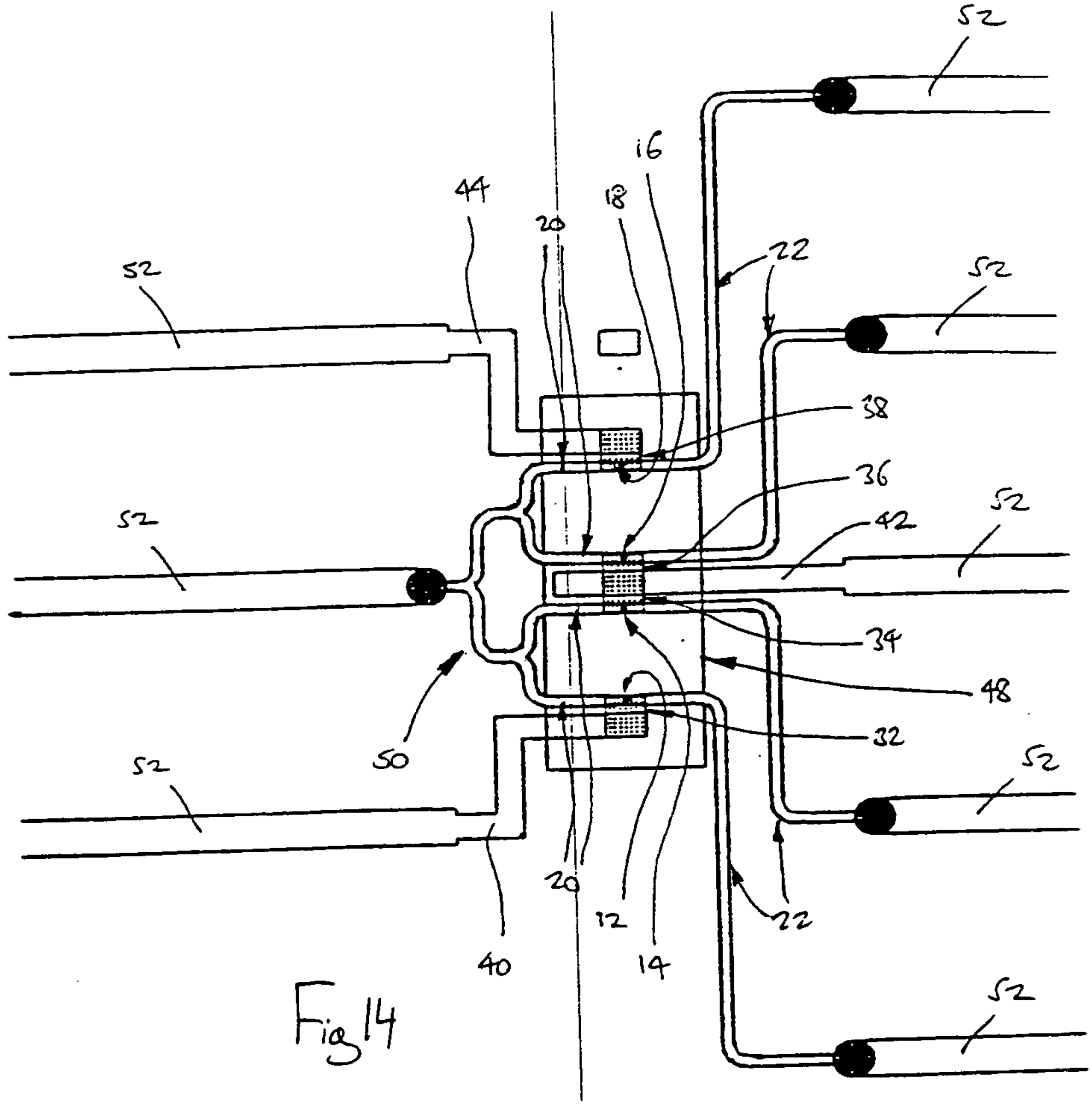

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Fig 14

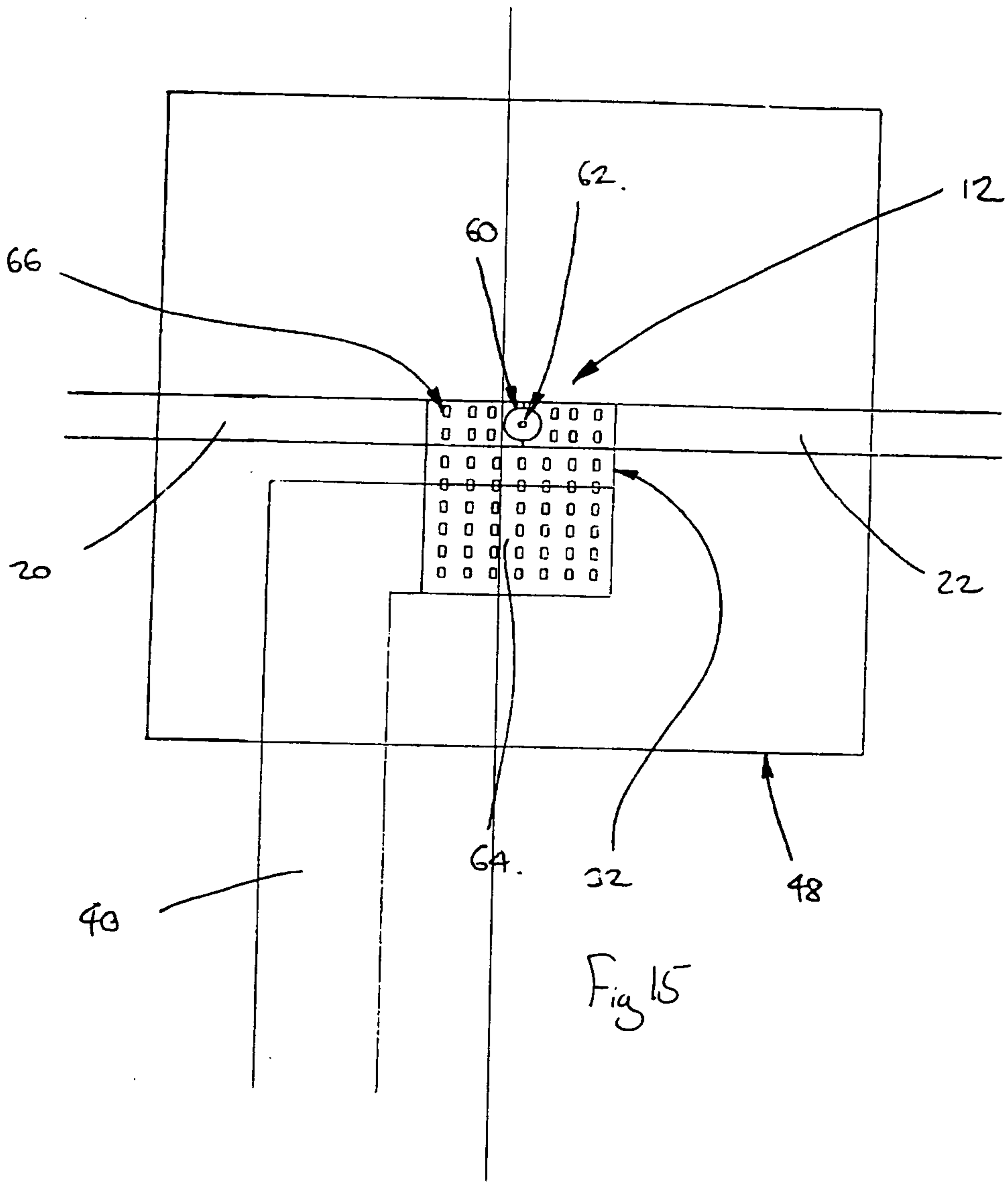


Fig 15

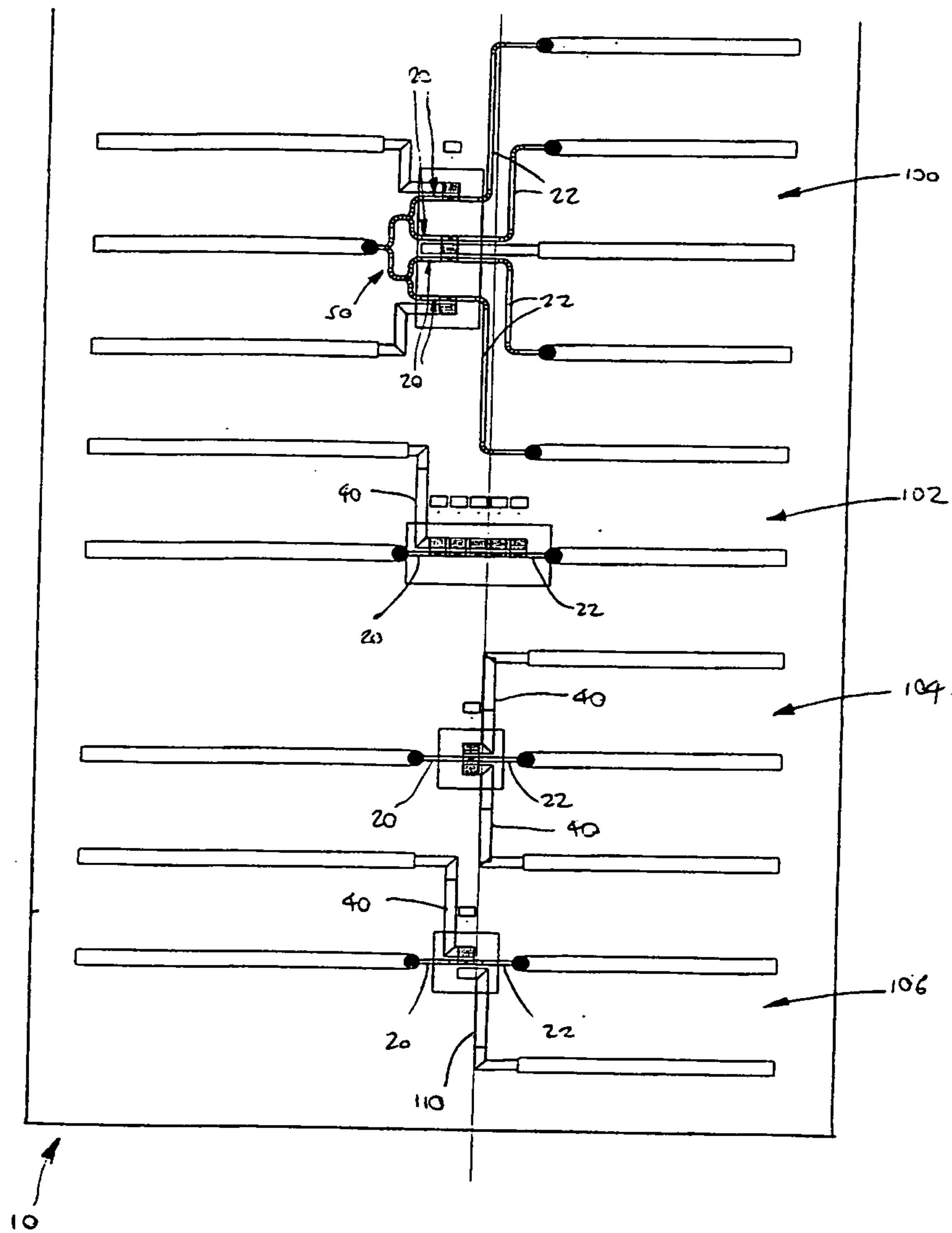


Fig 16

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03330

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C12M3/00 C12N15/89

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12M C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 5 457 041 A (GINAVEN ROBERT O ET AL) 10 October 1995 (1995-10-10) the whole document --- | |
| A | WO 96 10630 A (UNIV RUTGERS) 11 April 1996 (1996-04-11) the whole document --- | |
| A | WO 98 28406 A (BROWN DAVID ;DAVIS BRIAN (US); GENESYSTEMS INC (US)) 2 July 1998 (1998-07-02) the whole document --- | |
| A | WO 91 05519 A (US ARMY) 2 May 1991 (1991-05-02) the whole document ----- | |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 3 February 2000 | 10/02/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Hornig, H |

1

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Information on patent family members

International Application No
PCT/GB 99/03330

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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